LIPID AND CARBOHYDRATE SOURCES FOR ELEMENTAL, ENTERAL DIETS FOR NEONATES

By

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This dissertation is dedicated to my father,

Bernard Rudin,

who passed away in November, 1987. His humor, wisdom, support, and encouragement, unselfishly given to all who knew him, inspired great deeds. He believed that I could accomplish anything I set out to do. All things were possible.

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Colostrum-deprived piglets were fed either elemental, enteral diets, containing hydrolyzed whey proteins and malto-dextrins (Group E), or a conventional milk-based diet (Group C). Two litters were euthanized at birth (B0) to provide baseline values. Normal values were obtained from two litters of sow-fed (SF) piglets.

Group E piglets were surgically fitted with gastric, bladder, and umbilical artery catheters, and fed by bottle or continuous infusion pump. These piglets experienced diarrhea. Diet and flow rate modifications, made for each litter, reduced but did not eliminate the diarrhea.

To investigate diaccharidase activity in the small intestine (SI) of these piglets, tissue homogenates were incubated with maltose, lactose or sucrose. Maltase activity was low in the SI of B0 piglets suggesting that the malto-dextrin in the elemental diet was probably not well

digested by Group E piglets. Undigested maltose reaching the large intestine probably caused the diarrhea.

Maltase activity was detected in the SI of Group C and SF piglets even though they had never ingested maltose. Similarly, lactose activity was detected in the SI of Group E piglets that had never ingested lactose. Sucrase activity was very low in all of the piglets.

To investigate disaccharidase activity in neonatal foals, an oral tolerance test was conducted on 13 foals. Maltose, lactose, sucrose, and glucose solutions were administered orally on d 1, 3, and 5 postpartum. Changes in plasma glucose from venous blood were measured over time. Large peaks in plasma glucose occurred shortly after dosing with lactose and glucose on all days. This suggests that glucose from both substrates was absorbed. No rise in plasma glucose was detected after oral sucrose administration, even on d 5. Maltose was not well digested on d 1. However, some digestion may have occurred on d 3 and 5, as evidenced by small increases in plasma glucose after dosing with the maltose solution.

The colostrum-deprived piglet can be used successfully as an animal model for nutritional investigations. The results of the piglet and foal trials suggest that maltose and sucrose would not be suitable substrates for these neonates in the first few days of life.

CHAPTER I
REVIEW OF THE LITERATURE

Introduction

Advances in medicine have decreased greatly the mortality rate of weak or sick neonatal humans and animals. New techniques in neonatology, especially in the field of respiratory therapy, have enabled veterinarians to save many diseased or premature foals that otherwise would not have survived. The value, whether monetary or sentimental, of many horses today has resulted in a greater number of high risk foals receiving intensive care. As a result of the great strides made in saving these foals, a growing number of veterinary hospitals are developing or expanding their facilities for the care of neonatal foals.

Once conditions that immediately threaten the life of the newborn are stabilized, the primary concern becomes administration of nutrients in a form that can be readily utilized. In many cases, the foal can not or should not consume mare's milk. Some foals must be separated from their dams while they are in an intensive care setting. Sick or weak foals that can not stand to nurse must be hand-fed an appropriate enteral or parenteral diet.

Many of the feeding programs used on neonatal foals were originally developed for human infants in intensive care nurseries. Unfortunately, due to the difficulties in working with these neonates, many of the formulas and supplements being fed have not been well tested on very young or stressed foals (Koterba and Drummond, 1985).

Formulating Enteral Diets for Neonatal Foals

Before an appropriate diet for stressed foals can be formulated, basic research is needed to provide an understanding of their metabolism. Many questions about the efficiency of utilization of different nutrients remain unanswered.

Ensuring adequate caloric intake is of primary importance. In the past, some foals were inadvertently starved to death while being hand-fed because their energy requirements were underestimated (Koterba and Drummond, 1985). There is disagreement in the literature about the amount and type of enteral diet that will supply adequate nutrients during the critical neonatal period (Naylor and Bell, 1985).

Stressed or sick foals often have poorly functioning digestive systems and may not absorb nutrients or antibodies well. Some of these foals may even need parenteral feeding to increase the supply of nutrients available to the cells. Young foals are especially susceptible to infection when

they do not get an adequate supply of colostral antibodies from their mothers, or when they are premature or dysmature at birth (Rossdale, 1987). It has been shown in horses and in other species that the nutritional needs of premature and sick neonates can be different from that of term, healthy newborns. While their need for nutrients remains the same or even higher than that for healthy foals, their appetites may be poor (Koterba and Drummond, 1985). Therefore, the conclusions drawn from studies done on normal foals would not necessarily be applicable to the weak or sick patients treated in veterinary clinics.

Foals as Research Animals

Many problems present themselves when trying to design experiments involving a relatively large number of research foals. The expense of buying and feeding broodmares is great, and there is no assurance that each mare will produce a foal every year. Horses have a long gestation and tend to foal during the same time of year. This makes it difficult to spread foaling out over a long enough period of time to facilitate handling many foals.

The labor required for round-the-clock care of even a single foal is extensive. Even very young foals are relatively large and require more elaborate facilities than do small laboratory animals. Also, large radioactive carcasses resulting from studies involving labeled compounds

would be difficult to dispose of. These concerns tend to limit the number of foals that can be successfully studied each year. These problems can be overcome, in part, by using an appropriate animal model for preliminary investigations.

Using animals as experimental models for human studies is common practice. Ethical considerations have led to extensive use of animal models for research concerning human infants. Although the rat is frequently used as a model for adults, rat pups are not similar to infants or foals. Newborn rats have intestines that are fragile and difficult to work with, and the rats' stage of development and rate of growth at birth are very different from newborn humans, pigs, horses, and ruminants (Widdowson, 1984). Calves, while similar to foals, would not offer many advantages as a model for foals.

Piglets have been used successfully as models for premature infants. It has been suggested that they are closer to human newborns than any other animal (Glauser, 1966). One of the objectives of this research is to determine the appropriateness of using piglets as models for the neonatal foal.

Piglets as Models in Foal Research

One of the major limitations of any animal model is, of course, that the model is not going to behave exactly like the real subject. Therefore the model chosen should always come as close as possible to resembling the animal of interest. There are no references in the literature to previous work comparing piglets and foals, but information on each shows that these neonates share many common traits. The pig and the foal both tend to nurse frequently during the day for short periods of time (Pegorier et al., 1983). They have similar digestive functions at this age, both being adapted to a milk diet. Also, both species depend heavily on the passive immunity provided by the mother's colostrum, which must be ingested shortly after birth.

It has been shown by many workers that foals and piglets who do not acquire colostral antibodies are at severe risk from many diseases including pneumonia, septacemia, arthritis, and infectious diarrhea (Rossdale and Leadon, 1975; Naylor and Bell, 1985; Rossdale, 1985; Koterba et al., 1984; Mouwen, 1971; Bergeland and Henry, 1982). The colostrum from both sows and mares contains predominantly IgG (Pegorier et al., 1983; Naylor, 1979); however, IgA is present in greatest quantities in non-colostral mare's milk. Good management and sanitary conditions can help save hypoglobulinemic neonates, but the risk of infection is still high.

It has been suggested that mare's milk is closer to that of primates than any other animal, being high in lactose and low in fat and protein (Weaver, 1986). If mare's milk is similar to human milk, and piglets are used to simulate the response of infants, then perhaps the theory that foals can also be compared to piglets has some validity.

The use of the pig as a model has both advantages and limitations. Being able to do research on piglets that are littermates and on pigs from different litters, but by the same parents, allows experiments to be performed using large numbers of animals with minimal genetic variation. Also, many animals can be studied in a relatively short period of time, as the gestation of the sow (approximately 114 d) is much less than that of the mare (approximately 335 d). Sows can be bred to farrow all year, while mares have a foaling season of several months.

Digestive Problems of Neonates

After birth, mammalian neonates undergo a period of rapid acclimation to extrauterine life. They must adapt to using enterally rather than parenterally supplied nutrients. The neonate is now a free-living organism and it must be able to ingest and absorb nutrients from its environment. In mammals, of course, this usually means learning to nurse from its mother. Serious problems occur when the newborn has difficulty suckling or has an incompetent digestive tract. Immature or damaged intestinal tissues will not function normally, therefore, it is necessary to consider the nutritional needs of neonates with digestive problems separately from those of healthy neonates.

Diarrhea is a potentially serious problem for neonates and can occur for several reasons. The causes are usually classified as infectious or non-infectious.

Infectious Causes of Diarrhea

Diarrhea in piglets and foals has been attributed to an assortment of viruses and bacteria. Rotavirus, in particular, seems to be present in over 30% of the cases, while coronavirus and adenovirus are also common infectious agents (Palmer, 1985; Bergeland and Henry, 1982). These authors suggest that rotavirus acts by causing damage to the epithelium of the small intestine and leaving the neonate vulnerable to a secondary bacterial infection. The bacteria

usually cultured in cases of piglet diarrhea include Salmonella, and E. coli, while the latter is not considered a problem in foals. They also suggested that in foals, bacterial diarrhea may be secondary to septacemia and enteritis. Piglets may also be plagued by coccidiosis or infestations of strongyles.

Because of the ever present threat of invasion by infectious organisms, the area where newborns are housed must be kept extremely clean. It has been suggested that foals usually become infected through contact with adult, asymptomatic shedders on the same farm. Treatment for diarrhea in piglets and foals usually includes administration of antibiotics to cause a change in gut flora, supplemental vitamins to offset decreased gut absorption and bacterial synthesis, and, in piglets, antispasmodics (Mouwen, 1971; Palmer, 1985).

Non-Infectious Causes of Diarrhea

Stress of all kinds, including exposure to low ambient temperatures, or malfunctioning of the small intestine can predispose newborn animals to diarrhea (Bergeland and Henry, 1982).

Many foals suffer from diarrhea on d 6 to 14 postpartum. Because this is also the time when the mare returns to estrus after giving birth, it has been called "foal heat" diarrhea. However, there was no difference in

the amount or severity of diarrhea in hand-fed or mare-fed foals observed by Palmer (1985). Therefore, the theory that hormonal changes in the mare are reflected in the milk is unfounded. It is important, though, to monitor the foal closely at this time, because the onset of infectious diarrhea can mimic foal heat diarrhea.

Piglets may excrete thin, watery feces called "white scours" when they are very young. They usually seem normal in attitude, but may be less active. The feces, upon analysis, usually contains greater than 50% fat, but it is not known if this is the cause or effect of the condition. One experimenter suggests that it is the presence of abnormally structured gut villi that is responsible for the diarrhea (Mouwen, 1971).

Lipid Metabolism in Neonates

Fat in the Neonatal Diet

The addition of fat to human and animal enteral formulas has proven to be an effective means of increasing caloric density in an easily digestible form without also increasing osmolarity (Koterba and Drummond, 1985). By increasing the caloric density, the volume of formula to be fed can be kept low. Large fluid loads can be detrimental to sick or premature newborn patients. Fats are also added to enteral and parenteral solutions to provide essential

fatty acids and to provide a carrier for fat soluble vitamins.

Interest in studying the type of fat most readily utilized by neonates has increased because the composition of fat in fetal pig tissues and in sow's milk and colostrum can be influenced by the source of dietary lipids fed to the sow. Therefore, it may be possible to manipulate the diet of piglets to affect the compostion of body lipids. This is important because there is evidence that piglets metabolize fats differently according to chain length and degree of saturation (Miller et al., 1971; Lloyd and Crampton, 1957). This also appears to be true in human infants (Faber et al., 1988; Bach and Babayan, 1982; Lammi-Keefe and Jensen, 1984).

The fat content of milk and the extent of body lipid stores at birth vary with species. There is usually an increase in body fat of young animals after nursing. Neonates who are energy-deprived must depend on fat reserves for metabolizable energy and for thermal regulation (Pegorier et al., 1983).

It has been suggested that within 2 d of birth neonatal piglets can utilize fat as efficiently as glucose and the ability to use fat increases with age (Miller et al., 1971; Wolfe et al., 1978). Piglets have low fat stores at birth (approximately 1% of their body weight) that rapidly increase to about 15% of their body weight by day 14. Wolfe et al. (1977) fed isocaloric diets to colostrum-

deprived, newborn piglets. The diets differed only in the proportion of calories provided by carbohydrate and butterfat. The fatty acid pattern of the backfat of the piglets resembled the fatty acid composition of the diet, especially when high fat diets were fed. Lipogenic enzyme activity decreased with higher fat diets, which the authors suggest is due to the inhibiting effect of increased lipid intake rather than to decreased carbohydrate intake. Wolfe and coworkers in 1978 determined that on low fat diets, newborn piglets will resort to de novo fat synthesis and attain a level of fat deposition similar to piglets fed a high fat diet.

Fatty Acid Digestion and Absorption

Researchers have investigated the digestion and absorption of lipids in neonates of many species, but more information on how different types of fats are utilized by premature and compromised newborns is needed. Data on lipid metabolism in foals are limited.

Of particular interest is the effect of chain length on neonatal absorptive and metabolic processes. In the past, infant formulas contained exclusively long chain triglycerides (LCTs), but recent work suggests that compromised neonates may benefit from the replacement of some of the LCT with medium chain length fats. It has been suggested that medium chain triglycerides (MCT), usually

saturated fats consisting of 6 to 12 carbons, are readily hydrolyzed and absorbed by infants, rat pups, and piglets (Newport et al.; 1979, Putet et al., 1987; Jandacek et al., 1987).

Medium chain fatty acids (MCFA) from the diet are transported directly to the liver via the portal vein whereas long chain fatty acids (LCFA) travel through the lymph and then through the general circulation before reaching the liver (Newport et al., 1979). MCT being more polar and therefore more water soluble than LCT, are more easily attacked by pancreatic lipase and thus, are a desirable alternative to LCT in cases of pancreatic insufficiency or in other situations when LCT would be poorly absorbed (Jandacek et al., 1987).

Specifically designed triglycerides have been used to benefit from both MCT and LCT in a single lipid source. A triglyceride having medium chain fatty acids at the one and three positions and a long chain fatty acid at the two position may be the solution. This takes advantage of the pancreatic lipase specificity for cleavage at the one and three sites, leaving a 2-monoglyceride. Even in cases where LCT absorption is poor, the 2-monoglycerides seem to be easily transported across the intestinal mucosa (Newport et al., 1979), thereby avoiding essential fatty acid (EFA) deficiency. Human milk triglycerides have a 16:0 at the 2

position, which is thought to enhance the absorption of dietary fats (Lammi-Keefe and Jensen, 1984).

Metabolism of Medium Chain and Long Chain Fats by Neonates

The different metabolic pathways available to MCFA and LCFA affect the energy sources available to peripheral tissues. Interest in substituting MCT for LCT as part or all of the lipid content of enteral formulas stems from the products of metabolism of MCT. Several groups of investigators have begun feeding MCT to neonates in an effort to determine its benefits and drawbacks.

There is evidence to suggest that infants, especially preterm infants, do better on MCT-added formulas than on cow's milk alone, even though MCT are not found in large amounts in mature human milk (Putet et al., 1987). Lammi-Keefe and Jensen (1984) found considerably more 10, 12, and 14 carbon fatty acids in the milk of mothers that had very premature or premature babies when compared to the milk of mothers with full-term babies. They speculate that this may be of some special benefit to premature infants. Some researchers advocate feeding MCT to infants that have malabsorption of lipids (Bach and Babayan, 1982).

MCT and MCFA are not normally found in the blood even when they are added to the diet. They are thought to be rapidly metabolized by the liver and do not reach the

peripheral tissues intact. However, 15-20% of the fatty acids found in the umbilical cord blood of infants have eight carbons or less. MCT tend to increase carbohydrate tolerance, insulin secretion, and decrease glucose output from the liver when given orally (Bach and Babayan, 1982).

A study on neonatal puppies (Cotter et al., 1987) suggests that insulin causes LCFA to be removed from the blood for storage, while MCFA are removed for immediate use as an energy substrate. The authors have shown that low doses of MCT given intravenously (i.v.) are utilized more rapidly than LCT. The calories are available faster because the lipoprotein lipase (LPL) in the capillary walls quickly releases the MCFA, which then bind to albumin and are absorbed by peripheral tissues. In the liver, MCFA can enter the mitochondria rapidly, in contrast to LCFA which must be activated and be transported with the help of a carnitine carrier. MCFA can be oxidized so quickly that the Kreb's cycle is overwhelmed. When this happens, the 2-carbon units are funneled into ketone bodies which then diffuse into the blood. The authors suggest that MCT is a more flexible energy source than LCT. They also observed an increase in plasma clearance rate of long chain fats when medium chain fatty acids were present.

In a similar study, piglets fed either a 25:75 or 50:50 mixture of dietary MCT and LCT had higher blood glucose concentrations and increased insulin secretion than pigs fed

a diet in which the fat source was 100% LCT (Wolfe et al., 1978).

Newport et al. (1979) fed 2 day old neonatal pigs various ratios of MCT and LCT until they were euthanized at day 28. The pigs fed a 50:50 ration had similar growth rates to pigs fed 100% LCT as a dietary fat source. However, 90% MCT feeding resulted in a significantly slower growth rate. The pigs fed 90% or 50% MCT had higher liver weights but lower carcass weights than the 100% LCT group. The addition of MCT resulted in lower plasma lipid and free fatty acids (FFA), but higher body water, total body lipids, and cholesterol. They found no increase in nitrogen retention due to MCT feeding as had been suggested by others.

MCT feeding is not without possible disadvantages. Urinary output of dicarboxylic acids (DCA) may be seen. These end products of omega oxidation of medium chain fatty acids, such as seberic, sebacic, and adipic, are often detected in the urine during fasting, during ketosis, or in children with defects in fatty acid oxidation or carnitine metabolism. Henderson and Dear (1986) fed preterm infants averaging 29 wk gestational age either parenterally (no medium chain fats included) or enterally. The enteral formulas contained either a high amount of MCT (1.8g/100ml) or a low amount (.44 g/100 ml). Some infants received breast milk containing .15 g MCT/100 ml. The infants fed

the high MCT formula excreted larger amounts of DCA than infants fed the other feeding regimens, although no other symptoms were evident. It is unclear if this would be a problem for healthy infants. The authors stated that MCT feeding would be unadvisable in infants with β-oxidation defects and that more investigation is needed to determine the effects, if any, of increased urinary excretion of DCA. They did not comment on the possibility that DCA excretion resulted from inefficient use of MCT by the infants.

Ketones from MCT provide a quick source of energy for neonatal extrahepatic tissues. As their concentration in the blood increases, utilization by extrahepatic tissues increases. Therefore, they are a good substrate for energy production in times of high energy needs, such as growth or undernutrition (Bach and Babayan, 1982). Ketogenesis is controlled by a feedback system: when the concentration of ketones in the blood increases, negative feedback to the liver decreases production (Robinson and Williamson, 1980).

Gentz et al. (1970) have shown moderate increases in plasma ketones during starvation in newborn and 16 day old pigs during times when fat utilization is limited. They suggest that this may not be detrimental because 10 kg pigs are not prone to ketosis during starvation. Pegorier et al. (1983) question the extent of ketogenesis and β-oxidation in newborn pigs since they do not show hyperketonemia as do rabbits, rats, guinea pigs, and humans fed high fat diets.

In summary, the MCT system has a fast turnover rate but is easily overloaded. The presence of medium chain fats stimulate the absorption of plasma FFA. The 2-carbon units resulting from β-oxidation of MCFA are ketogenic, thereby providing the flexibility of an alternative energy source. The LCT system handles large quantities well but has a slow turnover rate. The resulting increase in plasma FFA may lead to acidosis. Some LCT are needed in the diet to prevent essential fatty acid deficiency. MCFA may improve the utilization and absorption of LCFA when there is impaired lipid digestion.

Disaccharidase Activity in Neonatal Small Intestine

Introduction

Milk is a complex mixture of nutrients, hormones, and other substances designed to provide optimal nutrition for a normal, healthy newborn. Consequently, it is a difficult task to formulate an artificial diet that will allow the hand-raised neonate to grow and develop normally. The challenge is even greater when the neonate is sick, weak or premature.

The developmental pattern of intestinal disaccharidases has been used to assess the nutritional status of neonates. It has been shown that improper nutrition results in a malnourished intestinal tract that does not show the same pattern of enzyme activity changes as a healthy neonate

nursing its mother (Rossi et al., 1986). These authors experimentally malnourished rats adding extra pups to a normal-sized litter. The pattern of disaccharidase activity in the malnourished rat pups resembled that of lesser developed pups. The maltase and sucrase activities were lower, and the lactase activity higher than in the control pups.

Sick neonates often lose the ability to digest lactose (Tzipori et al., 1984) and in infants, prematurity may result in low total intestinal lactase activity (Mayne et al., 1986). Therefore, it is important to assess the value of alternative carbohydrates when developing artificial formulas . Dairy farmers are also interested in the use of economical energy sources for use in calf milk replacers (Dollar and Porter, 1957). The efficient utilization of any energy source will depend on the activity of appropriate intestinal enzymes for digestion and absorption. Digestion of complex carbohydrates, such as corn starch, are cleaved by amylases into smaller units, into maltose, and finally into single glucose molecules that can be absorbed (Code, 1968). Therefore, starch can not be utilized unless there is adequate amylase and maltase present. Roberts (1974) detected only low levels of both heat-sensitive pancreatic α-amylase and heat-stable glucoamylase in neonatal foals. One way to determine the digestibility of various sugars for

use in neonatal formulas is to estimate the amount of disaccharidase activity present in the intestine.

Brush Border Disaccharidases

Disaccharidases are synthesized in membrane-bound polysomes within enterocytes of the small intestine. There is evidence to show that they are present in an inactive form in crypt cells, becoming activated when the cells have migrated to the top of the villus. Active lactase enzyme has only been found in the microvillus membrane on "mature" enterocytes at the tip of the villi in neonatal rats (Mackenzie, 1985). The enzymes are activated when proteases cleave a polypeptide chain from the large pro-disaccharidase molecule. Extracellular pancreatic proteases are responsible for activating maltase and the sucrase-isomaltase complex, while lactase is activated by intracellular proteases. The disaccharidases are attached to the brush border of the epithelial cells lining the intestine. In the rat, the rate of turnover for these large proteins is short, about 11.5 h. The enterocytes themselves take only 4 to 5 d to migrate from the crypt to the tip of the villus, where they are shed (Dahlqvist and Semenza, 1985). The rapid rate of cell division and protein synthesis makes these cells and their attached enzymes very susceptible to adverse conditions and poor nutrition.

Disaccharidase enzymes, primarily sucrase, maltase, and lactase, tend to follow the same pattern of development in most mammals, but the rate of development, pre- and post-partum, differs by species (Paige and Bayless, 1981). The amount of each disaccharidase present in the intestine will determine the carbohydrates that can be successfully incorporated into milk replacer formulas. Milk contains many substances that can affect the development of the intestines. Therefore, artificial formulas must be able to support the normal pattern of maturation seen in neonates nursing their mother. Determination of enzyme activity will allow assessment of the development and general health of the intestine. In this way, the nutritional value of artificial diets can be compared to that of milk and colostrum.

Developmental Patterns of Enzyme Activity in Neonates

The relative progression of the increase and decrease in disaccharidase enzymes is similar for many animals, but the timetables are very different for each species. For most mammals, lactase is high at birth, increasing during the nursing period, and decreasing near weaning. Lactase, which develops in human infants later in gestation than sucrase and maltase, reaches a peak at 36 to 40 wk gestation (Roggero et al., 1986). Therefore, very premature infants will have a limited ability to digest this sugar. Birth and

feeding each contribute to a postnatal rise in lactase in both term and preterm infants (Weaver et al., 1986).

Studies on neonates of several species indicate that prematurity or damage to the intestines can greatly reduce lactase activity. Mayne and coworkers (1986) developed a technique for assessing disaccharidase activity in living human infants. They demontrated a significant correlation between the enzyme activities measured in jejunal fluid with enzyme activities in adjacent mucosal tissue. Using this method, they studied eight premature, but appropriate for gestational age infants 17 times during the first 3 wk after birth. The infants were fed pasteurized breast milk via nasogastric tube. The lactase activity in these infants at 1 wk of age was lower than the activity present at 2 and 3 wk of age ($P < .001$). The 1 wk old infants had enzyme activities in the normal range for adults. This work agreed with other published reports indicating that lactase activity increases greatly near the end of gestation in humans. This results in a lowered capacity of preterm infants to digest a lactose-based diet.

Damage to the intestinal tissue may also result in reduced lactase activity. Biopsies were obtained proximally and distally to a complete or incomplete congenital blockage of the small intestines of 12 newborn infants during surgery (Serrano and Zetterstrom, 1987). The activities of all

disaccharidases in tissue samples proximal to the obstruction site were reduced when compared with previously published reference values. Lactase activity was more markedly reduced than the activities of the other disaccharidases. There was no difference in lactase activities in tissues from the four infants with complete blockages compared the eight infants with incomplete blockages.

However, when tissue samples from sites distal to the obstruction were compared, lactase activity was lower ($P < .03$) in infants with complete blockage when compared with infants with incomplete blockage. The authors suggest that distension and damage to the small intestine proximal to the blockages resulted in impaired development of brush border enzymes, particularly lactase. Minimal passage of ingesta through an incomplete blockage permitted greater development of lactase activity than detected in tissue distal to a complete blockage.

To investigate the effect of infectious pathogens on disaccharidase activities in neonates, Tzipori et al. (1984) inoculated seven 4 day old foals with various combinations of Streptococcus durans, Staphylococcus epidermis, and Escherichia coli isolated from feces of foals with naturally occurring diarrhea. Three foals received saline inoculations. Inoculations were adminstered via nasogastric tube and were followed with 200 ml evaporated

cow's milk. The foals were necropsied 1 to 3 d later. The seven foals inculated with pathogens had experienced diarrhea. S. durans bacteria were found to be adhered to the intestinal mucosa. This probably contributed to the significantly lowered lactase activity measured in these foals compared with the lactase activity of the control foals. These authors then conducted a similar study on newborn piglets, inoculating them with the same bacterial strains isolated from foals. These piglets also experienced diarrhea and reduced lactase activity compared with saline inoculated controls.

Maltase and sucrase do not appear in any appreciable amount until at least a few days after birth (Code, 1968; Veum and Mateo, 1986; Manners and Stevens, 1972; Ahrene et al., 1969). This is the case for rabbits, calves, pigs, rats, dogs, and cats (Code, 1968; Paige and Bayless, 1981). Guinea pigs, however, are very mature at birth, nurse infrequently, and can digest maltose and sucrose immediately (James et al., 1987). Human infants develop considerable maltase and sucrase activity before birth (Paige and Bayless, 1981).

It has been reported that newborn piglets can utilize lactose and glucose equally well at birth (Dollar et al., 1957; Ahrene et al., 1969), while sucrase and maltase activities increase during the first few days of life (Veum et al., 1986). Dahlqvist (1961a) reported high lactase

activity in the pig at birth and a rapid increase in maltase by day 2, but he found no appreciable sucrase at this age. In contrast, Bellis (1957) reported that by day 3, piglets could digest sucrose, lactose, and glucose equally well. James et al. (1987) found low levels of maltase and sucrase in sow-fed piglets on d 1 to 4 of life, with levels increasing from day 3 through day 10. Sucrase activity was twice that of maltase. When they gave Epidermal Growth Factor (EGF), which may be present in colostrum, to hand-raised piglets, they found an increase in sucrase and maltase in the middle and distal portions of the small intestine. EGF had no effect on lactase.

Dairy calf producers have tried to formulate milk replacers using economical energy sources other than lactose. Dollar and Porter (1957) fed newborn calves skim milk using starch as an energy source with and without amylase. Calves under 3 wk of age did poorly on this diet. The authors suggest that the calves were unable to digest the dextrins and maltose resulting from amylase digestion. They report only very low activities of maltase and amylase in the newborn calf and no sucrase activity. They state that calves under 4 wk old have only been shown to digest lactose and glucose. They did not see an appreciable rise in maltose digestion until the calves were 9 wk old.

Distribution of Disaccharidases

The distribution of disaccharidase activity in the small intestine has been investigated in several species. In calves, lactase activity is highest in the proximal small intestine. With age, lactase activity is reduce, mostly in the distal third (Huber et al., 1961). Manners and Stevens (1972) studied enzyme distribution in the newborn pig. While they found great variation from pig to pig, they were able to draw some conclusions. Enzyme activities were measured at various sites along the small intestine, with the proximal and distal ends represented as the 0% and 100% sites, respectively. Lactase activity was highest at the 20% site along the small intestine, decreasing to a low at the 95% site. From birth to 1 wk old, much of the decrease seen in lactase activity occurred in the proximal 50%, with higher activities found in the more distal section. Sucrase activity was only barely detectable until the pigs were 1 wk old, with the highest values in the proximal two-thirds of the small intestine.

In foals, lactase activity was reported to be highest in the duodenum and upper jejunum, decreasing distally (Roberts et al., 1974). Maltase activity was low in the equine fetus, increasing slowly during the first month after birth mostly in the proximal and middle segments of the small intestine, then increasing markedly until the end of the first year. Sucrase was very low at all sites along the

intestine in the newborn foal, increasing in the proximal sections during the first year, with the pattern of distribution parallel to that of maltase.

Postmortem studies of the entire length of small intestine of preterm and near-term human infants provided information on the development of disaccharidases (Raul, 1986). The relative distribution of sucrase and lactase was similar for infants in both age groups throughout the intestine. The greatest amount of lactase activity was found in the jejunum and duodenum, with little found in the distal small intestine. Term infants had five times greater lactase activity than the preterms, with the largest differences found in the proximal intestine. The greatest amount of sucrase activity was found in the jejunum. The term infants had more enzyme activity in all segments of the intestine. Glucoamylase was present in the tissues of preterm infants, increasing distally, as it does in adults. The authors suggest that this enzyme, along with salivary amylase (which, in preterm infants, is still functional in the small intestine) enhances digestion of glucose polymers.

Sucrase

Veum and Mateo (1986) fed pigs a sucrose-added formula but could not stimulate sucrase activity by day 7. No appreciable amounts of the enzyme were detected until day 14. Manners and Stevens (1972) found that pigs fed an

artificial diet had higher sucrase activity than sow-fed controls. This may suggest that diet influences the rate of development of the intestine. Huber et al. (1961) reported that even when calves were fed a sucrose-added diet, there was no notable digestion of sucrose until day 44, in contrast to their study with pigs, where sucrase digestion was evident by day 10.

Very low sucrase activity was detected in the small intestine of equine fetuses and newborn foals. The activity increased slowly during the first months after birth, reaching adult activities by 7 mo of age (Roberts, 1974).

Lactase

Humans are an unusual species. For some races, the ability to digest lactose remains long after weaning, but the percent of the population with adult lactase activity is small (Dahlqvist and Semenza, 1985). In humans, rabbits, and rats prolonged feeding or absence of feeding of lactose does not affect the postnatal changes in lactase activity (Paige and Bayless, 1981). In calves, up to 44 d of age, no increase in lactase activity occurs as a result of adding lactose to the diet (Huber et al., 1961).

In human infants, lactose hydrolysis by the β-galactosidase enzyme is affected by other mono- and disaccharides that may be concurrently ingested (Paige and Bayless, 1981).

Lactase activity in the neonatal piglet has been shown to be high at birth, reaching a maximum at day 15, then decreasing throughout the nursing period with low activities still detectable in the adult (Paige and Bayless, 1981; Veum and Mateo, 1986; Manners and Stevens, 1971; Dollar et al., 1957; Dahlqvist, 1961b).

Lactase activity was detected in the mucosa of equine fetuses as early as 105 d of gestation, increasing after the ninth month, reaching a maximum at birth and remaining high for 4 mo (Roberts et al., 1974). The activity declined, especially in the duodenum, until 1 yr of age.

Maltase

Several researchers have investigated maltose as an energy source for neonates. Cunningham and Brisson (1957) fed 1 day old piglets a purified casein-based diet with either maltose or glucose as an energy source. In piglets 3 to 7 d old, greater than 95% of the maltose in the diet, and greater than 99% of the glucose disappeared from the small intestine. The glucose diet resulted in greater digestibility for protein and carbohydrate than the maltose diet ($P < .01$). When the entire small intestine of neonatal piglets of various ages was ligated and injected with a 5% maltose solution, .66 μmol of maltose was hydrolyzed and absorbed per h per kg body weight. This increased to 1.05

μmol by day 6 and 7. The authors conclude that maltose digestion during the first wk may only be borderline in supporting the animal's energy needs, but after day 7 maltose is used just as efficiently as glucose. Huber et al. (1961) could not increase the maltase activity of calves by feeding diets of whole milk plus lactose, or sucrose and starch for 44 d. They note that this is in contrast to other workers who have reported an increase in maltase activity beginning in the third or fourth wk after birth.

Using intestines obtained from slaughterhouses, Roberts et al. (1974) detected low maltase activity in equine fetuses during the first 3 mo of gestation. Activities remained low until birth. Enzyme activity doubled during the first month, with the greatest increase occurring in the proximal small intestine. Adult activities were achieved by 7 mo of age. No data were given specifically for the early neonatal period.

Determining Disaccharidase Activity in Experimental Animals

Intestinal disaccharidase activity can be determined by homogenizing samples of tissue from segments along the small intestine and incubating the homogenate with disaccharide substrates. The glucose produced is a measure of enzyme activity. Many researchers have used this method, as modified from Dahlqvist (1964). The major disadvantage of this method is that the experimental animal must be euthanized. Economic constraints usually limit the number of foals that can be euthanized, making it impossible to conduct large scale equine trials of this type. Therefore, it would be helpful to have an alternate method, at least during preliminary trials. Studies on calves have shown oral tolerance tests to correlate well with the results obtained using tissue homogenates when comparing relative enzyme activities (Dollar and Porter, 1957).

Roberts (1975) used an oral disaccharide tolerance test to determine the digestibility of various sugars in adult horses and ponies. Although foals were not included in the study, Roberts suggested that an oral lactose tolerance test would be a useful tool for determining digestive competence in the sick or premature foal. Testing the digestibility of other sugars (for possible inclusion in artificial formulas) in this way would allow animals to be studied without euthanasia.

Responses of the Neonate to Stress

Stress factors include a wide variety of situations that can cause problems for neonates. Poor maternal nutrition and dystocia are examples of stresses can occur before or during birth, but this project will be concerned with stresses that occur during the neonatal period.

Failure to Acquire Passive Immunity

In the horse and pig there is little transport of immunoglobulins across the placenta. So, these species must depend on colostral transfer of antibodies to protect them from a wide variety of infectious organisms until their own immune systems are competent. The risk is high that these neonates will be severely stressed by infections if they are deprived of colostrum or are unable to absorb antibodies during the first day of life. This becomes even more serious if the neonate is also exposed to other stresses in its environment or is weak, sick, or immature at birth.

Many researchers have emphasized the importance of colostrum ingestion for newborn foals and have suggested reasons why foals may be hypogammaglobulinemic even after they have nursed (Naylor and Bell, 1985; Naylor, 1979; Rossdale, 1985). Their findings show that immunoglobulins in the colostrum decline rapidly, becoming 15% of its concentration at parturition in 4 to 8 h.

Mares may produce poor quality colostrum for several reasons. Older broodmares may leak colostrum before parturition and so lose the high antibody content necessary to provide the foal with adequate protection. Some foals are born prematurely, before the mammary gland has completed concentrating the immunoglobulins. Foals must ingest colostrum within 24 h of birth. After this time, the digestive tract "closes" and will no longer absorb large molecules. Closure is not delayed by food-withholding.

Foals may be deprived of colostrum if they are orphaned or because of a problem with the mare or with the foal itself that prevents it from nursing. Foals that need to be hospitalized may be separated from their mothers. Frozen colostrum may either be unavailable or unwise to feed, depending on the condition of the foal. Colostrum-deprived foals have been shown to remain almost completely agammaglobulinemic for 2 wk, then begin rapid synthesis of antibodies until they appear the same as colostrum-fed foals by 5 wk of age (Naylor, 1979). These workers feel that over 50% of colostrum-deprived foals will develop an infection resulting in septacemia, diarrhea, pneumonia, omphalophlebitis, or arthritis within 1 wk of birth. They suggest prophylactic treatment of 1.2 liters of plasma taken from an adult horse, given intravenously over 2 h, to supply an adequate amount of IgG.

Piglets are also likely to suffer similar diseases if not provided with passive immunity from colostrum (Svendsen and Bille, 1981; Bergeland and Henry, 1982) or kept in isolated, aseptic conditions (Pond, 1978). In addition to providing passive immunity to neonates, colostrum also contains epidermal growth factors that promote mucosal development in the gastrointestinal tract (Svendsen and Bille, 1981). Unfortunately, it has been shown that colostrum-deprived neonates fed either milk from other species or soy-based formulas sustain damage to the intestine, as the immune system responds to the foreign proteins ingested (Weaver, 1986). The use of elemental diets would prevent this adverse reaction as they contain hydrolyzed rather than whole proteins.

Cold Stress

Exposure to a low ambient temperature shortly after birth is a common problem for many neonates including piglets, foals, and human infants. Management practices used on many breeding farms encourage mares to begin cycling and ovulating earlier in the year than they would in nature, so foals are born more and more frequently in late winter/early spring when temperatures are low. Therefore, it is important to study the ability of neonates to regulate their body temperature and the effects of cold stress on their health and nutritional requirements.

Normal body temperature for foals is 38.05^{O}C ±1, with the lower boundary of the thermal neutral zone being 14^{O}C during the first 48 h postpartum. After that, the foal can tolerate an ambient temperature as low as 8^{O}C, provided he has nursed and the environment is free of draught (Rossdale, 1985). Premature foals often have difficultly in maintaining their body temperature, which may drop to 36.6^{O}C or even lower (Naylor, 1979). Foals lack significant deposits of brown adipose tissue and must rely on shivering for thermogenesis (Rossdale, 1985).

The normal body temperature of piglets is 39^{O}C at birth. The thermal neutral zone is very small (34-35^{O}C) (Stanton et al., 1973) and they have greater difficulty maintaining their body temperature under cold stress than do foals and calves (McCance and Widdowson, 1959).

Piglets are easily cold stressed for several reasons: they are poorly insulated at birth, having almost no brown adipose tissue and only 1% of their body weight (about 10 g) is fat; they have a small body size with a large surface area; their metabolic rate is low at birth; and they are born with no protective hair or fur (McCance and Widdowson, 1959; Gentz et al., 1970; Curtis and Rogler, 1970; Stanton et al., 1973). However, their ability to thermal regulate does increase with age and is fairly well developed by 1 wk of age (Gentz et al., 1970; Curtis and Rogler, 1970; Stanton et al., 1973).

Piglets will experience a 2 degree decrease in body temperature, to 37^{O}C, shortly after birth. Adverse effects may be seen if the temperature reaches 35^{O}C (Svendsen and Bille, 1981).

An ambient temperature of 5^{O}C is close to the cold limit for newborn pigs. At this temperature, there should be maximum utilization of their thermal regulatory capabilities. Several investigators have exposed newborn pigs to this temperature for short periods of time. Results suggest that starving piglets undergoing cold stress experience hypoglycemia and will not survive longer than 30 h unless fed (McCance and Widdowson, 1959). These workers report that at birth, pigs are able to increase oxygen consumption during cold stress whether they are fed or not, whereas the newborn rat will not show an increase until it is 5 d old. Rabbit young improve their ability to respond during the first few days of life.

Carbohydrate is the main energy source of newborn piglets, even though sow's milk contains 30-40% fat on a dry matter basis (Allee et al., 1971) because fat utilization is limited during the first few days postpartum, regardless of the ambient temperature. However, cold exposure results in rapid depletion of energy stores and death from hypothermia unless the piglets are fed. It is estimated that the carbohydrate and fat stores endogenous to the piglet would

yield about 72 h worth of energy with ambient temperatures in the thermal neutral zone (Gentz et al., 1970).

Compromised Neonates

Being born premature or dysmature will compromise the neonate's ability to adapt to extrauterine life. Dystocia and neonatal maladjustment syndrome (where there is reduced ability to suck, swallow, or move about) also predispose newborns to septacemia and in the case of foals, septic arthritis (Koterba et al., 1984). Premature foals usually have abnormal blood gases and an impaired acid/base status (Rossdale et al., 1987). They often are weak and take a longer-than-normal time to stand and nurse. They commmonly show hypoflexion of the metacarpal and metatarsal joints, and a bright red tongue, but they may maintain normal respiration and heart rate for the first 24 h before their condition deteriorates (Rossdale, 1987).

Intestinal maturation will not be complete in preterm neonates. Enterocyte function, villi development, and enzyme activity are often different from that found in term newborns, resulting in decreased digestibility of some nutrients (Raul et al., 1986; Mayne et al., 1986; Mackenzie, 1985).

Hematology and Blood Chemistry of Neonates

Hematology

It has been suggested that evaluating the packed cell volume (PCV), hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), and differential leukocyte count can be helpful in assessing the condition of neonates and estimating the degree of maturity and viability of neonates (Rossdale, 1985).

Becht and Semrad (1985) found that in foals, PCV, Hb, and RBC counts peak at birth and then begin to decrease within 12 h postpartum. They also noticed that mean corpuscular volume (MCV) decreased slightly in the fetus just before birth, then remained steady for the first 2 wk of extrauterine life. They suggest that some of these indices may be useful for establishing the maturity of the foal at birth.

Several workers have reported lower leukocyte counts (4×10^9/liter) for premature foals as compared to full term foals (6×10^9/liter). Erythrocyte counts were also lower (6×10^{12} vs 12×10^{12}/liter) as was the PCV (33 vs 45.6%). Globulin (<10 gm/liter) and gammaglobulin (4 gm/liter) were also lower than for normal foals (Jeffcott et al., 1982; Kitchen and Rossdale, 1975; Rossdale, 1983; Becht and Semrad, 1985).

Foals have an intact granulocytic system at birth. The increase in WBC reported during the first day of life is

thought to be due to an increase in mature polymorphonuclear neutrophils (PMN) and lymphocytes. The increase in PMN continues during the first 3 days postpartum. During the first wk, monocytes begin to appear. Basophils are not normally seen in the neonatal period.

Premature foals tend to have lower WBC (4×10^9/liter), RBC (6×10^{12}/liter), PCV (33%), globulin (<10gm/liter), gammaglobulin (4gm/liter) and a narrower neutrophil to lymph ratio (1.2:1) than normal foals. A severe leukopenia, left shift, and the appearance of toxic neutrophils may indicate sepsis (Becht and Semrad, 1985).

Jeffcott et al. (1982) and Rossdale (1983) found that premature foals consistently show a lower than normal neutrophil to lymphocyte ratio (N/L). Normal foals have a ratio > 2:1 while premature foals show a reversed N/L ratio of > 1:1. A dose of short acting exogenous ACTH will elict a neutrophilic response in mature foals but not in immature foals that lack normal adrenal function.

In piglets, RBC (from 6.18×10^{12}/liter to 4.4×10^{12}/liter), PVC (from 40 to30%), and Hb (12.5 to 10.0g/dl) are decrease during the first wk after birth. However, the number of WBC tends to increase from about 6.2 to $17 \times 10^3/\mu l$. The proportion of PMN to lymphocytes percentages shifts from about 38:60 to about 53:42. Granulocytes are rarely seen during the first wk (Schmidt and Tumbleson, 1985).

Chemistry

Various clinical chemistry analyses are useful for evaluating the general health and nutritional status of newborn animals. Both foals and piglets are susceptible to hypoglycemia shortly after birth, especially if they are weak, sick or stressed.

Normal blood glucose in the neonatal piglet is 60 to 80 mg/dl. However, it may be as high as 100 mg/dl immediately after birth. The decrease may be due to limited glycogen stores in the liver (Pond, 1978). Blood glucose values as low as 48 mg/dl have been reported for newborn pigs, increasing to 114 mg/dl by day 7.

Foals are susceptible to hypoglycemia shortly after birth, especially if they are weak, sick or stressed. Foals that have suckled during the first 2 h after birth were reported to have the following blood glucose values: 95.5 $\pm$ 17.44 mg/100 ml at birth, 83 $\pm$ 3.28 mg/100 ml 30 min postpartum, and 131 $\pm$ 12.4 by 14 h (Kitchen and Rossdale, 1975). Normal, healthy foals that have suckled have blood glucose levels that are higher than adult levels for the first 24 h and remain in the high normal adult range for the first 30 d. Hypoglycemia may result in sick foals not receiving sufficient nutrient intake, or from poor digestive and absorptive function even with adequate intake (Becht and Semrad, 1985).

Blood lipid components such as free fatty acids, trigycerides, ketones, blood urea nitrogen (BUN), and circulating liver enzymes are important parameters to consider when evaluating the utilization of dietary fat and nitrogen status in the neonate.

Milk Composition and Intake

Some researchers, using milk replacers currently available for foals, have expressed dissatisfaction with the foals' growth rate and general appearance. They report that foals fed milk replacers according to directions usually remain small (Naylor and Bell, 1985). Since no other farm animal species produces milk similar to that of the mare, finding a suitable formula is more complicated than just substituting cow's milk, for example, for mare's milk. However, goat's milk has been used successfully to raise orphan foals. It is higher in fat and protein and lower in carbohydrate than mare's milk, but foals drink it readily and thrive on it, although some may experience diarrhea (Koterba and Drummond, 1985). Interestingly, goat's milk is similar to sow's milk, so perhaps this would further suggest that piglets would be good models for foals.

Even though goat's milk and colostrum contain slightly more protein and much more fat than that of the mare, foals have been successfully raised at the veterinary teaching hospital at the University of Florida on goat's milk

(Koterba and Drummond, 1985). Goat's milk is high in fat and is similar to sow's milk (Glauser, 1966). Also, many commercially available milk replacers for foals are fed at twice the recommended concentration, which significantly increases the fat intake (Naylor and Bell, 1985).

The compositions of colostrum and early lactation milk from mares and sows are compared in Table 1.1. Ullrey et al. (1966) and Pagan and Hintz (1985) reported data on equine milk constituents, while Widdowson (1984) and Widdowson (1985) reported on both sows and mares. While similar in protein, sow's milk has higher total solids, higher fat, and lower lactose than mare's milk.

Milk Production in Mares

Compared to other species, mare's milk is unusually high in water. To compensate for this, foals must consume large quantities of milk and have a rapid body water turnover rate (the halflife of body water for foals under 1 wk old is 2.5 d). From birth to 11 d, foals will usually drink about 16.2 kg of milk per day, ingesting about 422 g protein and about 9830 kcals (Palmer, 1985).

When mares were fed iso-caloric diets that differed only in the proportions of fiber and fat, milk compositions did not differ (Pagan and Hintz, 1986). Mares fed diets that contained 1.25 times the National Research Council

Table 1.1. Comparison of colostrum and milk produced by sows[a] and mares[b].

Milk Constituent	Mare	Sow
% Dry Matter	11.6	20.1
% of Dry Matter:		
FAT	15.0	42.0
PROTEIN	22.8	29.0
CARBOHYDRATE	58.8	24.0

Colostrum Constituent	Mare	Sow
(gm/100ml milk)		
PROTEIN	25.2	17.8
FAT	.7	4.4
LACTOSE	4.6	3.5

[a]Widdowson, 1984
[b]Ullrey et al., 1966

(NRC) recommendations for energy requirements for lactation produced greater volumes of more dilute milk than did mares fed adequate calories. The growth rates of the foals were not different for the two groups. The authors concluded that feeding excess energy does not improve foal performance, and results in obese mares.

Special Nutrient Requirements of Neonates

It is is important to determine the special nutrient needs of neonates when developing a suitable enteral formula. Premature neonates, for example will have different nutrient requirements from healthy, full term newborns (Koterba and Drummond, 1985). These authors suggest that besides the major nutrients, requirements for folate, Vitamin E, cysteine, calcium, and phosphorous requirements for premature foals need to be evaluated. They have found that stressed foals require about 120 kcal/kg/d and may be expected to consume 20-28% of their body weight (BW) in milk per day. They also recommend feeding an elemental type of diet, because stressed newborns may not tolerate milk-based diets which can cause bloat, colic, and diarrhea. Elemental diets may prove to be a good alternative to feeding cow's milk or soy-based formulas that have been shown to have damaging effects on the intestinal villi of infants (Weaver, 1986).

By examining prepartum mammary gland secretions, we may be able to determine which nutrients are of special benefit to premature neonates. In humans, the mammary gland produces considerable amounts of medium chain fatty acids (C8-C14) after parturition (Bitman et al., 1986). Linoleic (18:2) also increases when compared to prepartum mammary gland secretions. The secretion of several long chain polyunsaturated fatty acids (PUSFA) decreases. Prepartum secretions contained large amounts of C16 and C14 when compared to normal milk. After parturition, the milk contained increasing amounts of short and medium chain length fatty acids (4:0, 6:0, 8:0, 10:0) as well as 18:0 and 18:1. Similar patterns of increased lipid synthesis and a shift towards medium-chain fats after parturition were also observed in cows. These authors suggest that the increase in MCT in the milk may be of some advantage to preterm neonates whose capacity for lipid digestion may not be completely matured.

CHAPTER II
EVALUATION OF AN ELEMENTAL, ENTERAL DIET FOR NEONATES USING PIGLETS AS A MODEL

Introduction

As recently as 20 yr ago, premature or sick human infants were not expected to survive the neonatal period and often were allowed to starve to death (Koterba et al., 1985). Today, however, the advent of improved respiratory therapy, refined surgical techniques, neonatal intensive care facilities, and specially trained personnel gives the very low birth weight or compromised infant a dramatically improved survival rate. Once these infants could be kept alive for longer periods of time, the question of nutrition became important.

The field of equine neonatology, which has expanded considerably over the past few years, has benefitted from the advances made in human neonatology. Today, premature and sick foals are treated successfully using much of the same equipment and techniques developed for infants. Many of these foals have gone on to achieve success on the race track and in the show ring. Veterinarians are now faced with the same questions faced by neonatologists treating

infants. Now that life threatening infections and respiratory problems can be overcome (Webb et al., 1984; Baker and Ames, 1987) what type of nutrition is appropriate for these patients?

Ethical considerations make some types of research impossible on human infants. Animal models have proven useful in situations where invasive techniques or use of radioisotopes is necessary. It was suggested that neonates of different species may be more similar to each other than they are to the adults of their species (Koterba et al., 1985). Therefore, it is possible to use information gained from work in one species for use in another. For example, Rossdale (1987) used the human neonatology terms premature, dysmature, and small for gestational age, to develop guidelines for assessing readiness for birth in newborn foals.

Although the elemental, enteral diets used in this trial was designed originally for use in humans, scientifically controlled trials on neonates were needed. It was thought that this formula might be suitable for premature or sick foals which were unable to drink mare's milk. These compromised neonates may have poorly functioning or damaged intestinal tracts.

Nutrients in enteral diets are delivered to the digestive tract usually by mouth or by catheters inserted in the stomach or small intestine. In contrast, parenteral

formulas are delivered intravenously. In an elemental diet, individual nutrients are added in easily digestible forms, in contrast to conventional, milk-based formulas. The base formula tested in this study contained hydrolyzed whey proteins rather than whole milk proteins. The formula was lactose-free because, as stated previously, lactase may be deficient when there is damage to the intestines. Milk-based formulas contain long chain lipids almost exclusively, but this formula included some medium chain fats. This was because the digestion, absorption, and metabolism of long chain fats may be inefficient in compromised neonates.

Piglets were used as a model for the preliminary research in this trial. The information gained from these trials would then be used as the basis for studies on foals and infants. Colostrum-deprived piglets were used for several reasons. If the piglets were allowed to nurse the sow, it would not be possible to ensure that each piglet received the same amount of colostrum. By hand-feeding the piglets from birth, the amount and type of each nutrient consumed can be controlled. Many sick neonates do not receive colostrum shortly after birth and so are deprived of its growth-promoting factors. The colostrum-deprived piglet model permits the formula to be tested on stressed animals. The restrictions and limitations of research projects with foals and infants encourages the use of an appropriate animal model for all preliminary work.

This study was part of a multi-project program to develop an enteral diet for premature or sick foals. Designing such a diet is a complex task requiring a series of separate trials to determine the ideal formulation.

The goals of this specific study were:

1. to learn techniques and methods used in the investigation of neonatal nutrition using a piglet model;
2. to determine the effect of an elemental, enteral diet on the growth, intestinal development and general health of colostrum-deprived neonatal piglets and, secondarily, to learn to formulate diets with different lipid sources;
3. to evaluate and improve surgical and daily care procedures for colostrum-deprived piglets hand-raised for 7 d; and,
4. to use the information obtained from the piglet study towards possible development of a diet for neonatal foals.

Materials and Methods

Animals

This study was conducted using newborn Yorkshire x Hampshire x Duroc crossbred piglets. The sows were bred and cared for by the staff of the Reproductive Physiology Unit under the direction of Dr. Fuller Bazer of the Animal Science Department. Labor was induced 2 d prior to the expected farrowing date to ensure that the birth would be attended. The sow was injected with Prostaglandin $F_{2\alpha}$ at

730 h on d 111 of gestation. Oxytocin was administered at 900 h on d 112. Farrowing usually occurred within several hours after the oxytocin injection. All piglets were removed from the sow immediately after birth, before nursing. The umbilical cord was tied and cut. Identifying markings, sex, weight, and general condition were recorded. Only piglets greater than 750 g were admitted to the trial. The protocol that subsequently was followed depended on the treatment assignment of the litter.

Piglets from a total of 10 different litters were used for the trial. Only one litter was on trial at any one time due to time, labor, and facility constraints.

Baseline values were obtained from 18 piglets from two litters removed to the animal facilities at the Food Science and Human Nutrition Building and euthanized within a few hours after birth. These non-fed piglets are referred to as birth piglets (B0).

Normal values for sow-fed animals (SF) were obtained from 22 piglets from two litters were returned to the sow and allowed to nurse for 7 d, after which they were euthanized. The sow and piglets were housed in an environmentally controlled room. The sow was confined to a farrowing stall of pipe construction. The piglets could enter the stall easily under the bottom pipe or retreat out of the stall to a corner of the room to avoid the sow. Rubber floor mats were used to reduce conductive heat loss

and improve footing. Clean hay was provided for bedding in a corner of the room where the piglets often slept. A heat lamp provided additional warmth. The SF were monitored and weighed daily.

Thirty-eight experimental piglets from six litters were transported to the Piglet Neonatal Intensive Care Unit (PNICU), an environmentally controlled room (37°C, 70% humidity) similar to hospital nurseries designed for human neonates. These colostrum-deprived piglets were hand-raised in individual plexiglass boxes (measuring 14" x 14" x 17") in the PNICU for 7 d.

Surgery

All experimental piglets except those bottle-fed the milk-based diet underwent surgery. Umbilical artery and bladder catheters were inserted to permit collection of blood and urine, respectively, during the trial. The samples were used to evaluate the health of the piglets. Gastric catheters were inserted to permit feeding by constant infusion pump.

Development of anesthesia procedures. Piglets were anesthetized for surgery with intramuscular injection (75 mg/kg BW) of ketamine HCl. Lidocaine was given subcutaneously at the incision site. Once an intravenous (i.v.) catheter was placed in the umbilical artery, an Acepromazine drip was started at 60 drops/min. Full

recovery from this anesthesia was slow, delaying the initiation of enteral feeding.

In an effort to decrease recovery time, the last two litters on the trial were given the inhalant isoflurane by mask as the sole anesthetic. Schieber et al. (1986) found that although isoflurane caused a reduction in blood pressure, peripheral resistance was reduced, preventing a decrease in cardiac output. These workers suggest that isoflurane, well tolerated by newborn piglets, has a clear advantage over the use of halothane. The piglets in the present study tolerated isoflurane well, recovering quickly (some were quite active within minutes of removal of the inhalant).

Catheter placement. Each piglet was fitted with an umbilical artery catheter for administration of i.v. fluids (.45% saline and 5% dextrose) during and after surgery, and for collection of blood on d 3 and d 5 of the trial. The piglets received the i.v. fluids until they were fully recovered from the anesthetic and enteral feeding was initiated, at least 12 h post-surgically.

Piglets also were fitted with bladder catheters for urine collection. The ureters were left open to permit normal urination.

The introduction of gastric catheters and the use of continuous infusion pumps allowed enteral feeding to be

All catheters were externalized through a single abdominal incision.

Post-operative care. After surgery, the piglets were returned to their plexiglass boxes in the PNICU and observed closely. A chart was kept beside each box for recording data from individual piglets. Respiration, temperature, color, activity level, and urine output, were recorded every 30 minutes until the piglets had recovered fully.

Diets

Hand-raised piglets were fed one of three enteral formulations. A conventional, cow's milk-based formula was bottle-fed to a total of 12 piglets from two litters. These piglets, designated Group C, did not undergo surgery. In addition, four piglets were fed this formula via gastric catheter. Previous trials had shown that the growth rate of piglets fed this formula was similar to the rate of sow-fed piglets.

The two other diets used in this study were based on the liquid elemental formula Peptamen (Carnation). All piglets fed diets containing the elemetal formula were designated Group E. Hydrolyzed whey proteins provide amino acids and malto-dextrin is the carbohydrate source. The formula was fed in two different, isocaloric forms based on the predominant fat source. These were designated as MCT (medium chain triglyceride) or LCT (long chain triglyceride)

the predominant fat source. These were designated as MCT (medium chain triglyceride) or LCT (long chain triglyceride) formulas. Because Peptamen is much higher in carbohydrate than sow's milk, it was diluted to one-half concentration before use. However, this necessitated the addition of whey (27.4 g/liter for the MCT formula and 35 g/liter for the LCT formula), calcium citrate (4.38 g/liter), and calcium phosphate (5.77 g/liter) to obtain a composition similar to sow's milk. The wrong type of whey mistakenly was added to the formulas fed to one of the litters, invalidating the data obtained from these pigs. The diets resembled sow's milk in the amount of fat (60 g), protein (75 g), and carbohydrate (63.5 g) provided per liter. Carnitine was added at 486 μl/liter of formula. The MCT formula was obtained by adding 15 g medium chain triglyceride oil (MCT Oil, Mead Johnson and Co., Evansville) and 6 g sunflower oil (SFO) to 1 liter of Peptamen. All of the lipids in SFO are long chain fats. The final MCT:LCT ratio was 70:30. The LCT formula required the addition of .8 g MCT oil and 51.8 g SFO to 1 liter of low-fat (LF) Peptamen. This resulted in a 90:10 LCT:MCT ratio. The composition of sow's milk and the dietary treatments used in this trial are presented in Tables 2.1-2.2.

Table 2.1 Composition of dietary treatments, base formulas, and sow's milk on a per liter basis.

Diet	Composition							
	Protein		Fat		Carbohydrate		Calcium	Phosphorus
	g	kcal	g	kcal	g	kcal	g	g
Sow's milk	72	288	61	549	48	192	2.1	1.9
Peptamen (U-5122-1)[a]	40	160	39	351	127	508	0.6	0.5
Low Fat Peptamen (U-5216)[b]	47.6	190	7.4	67	186	744	0.6	0.5
MCT Formula	75	300	60	540	63.5	254.5	2.5	2.0
LCT Formula	75	300	60	540	93	372	2.5	2.0

[a]Base formula used for MCT diet.

[b]Base formula used for LCT diet.

Table 2.2 Composition of sow's milk (Pond and Houpt, 1978) and Peptamen (Carnation) on a per liter basis.

Nutrient	Sow's Milk	Peptamen
Protein, g	72	40
Fat, g	61	39
Carbohydrate, g	48	127
Folic Acid, g	3900	4000
Thiamin, g	650	1500
Riboflavin, g	1370 - 8200	1700
Niacin, mg	4.3 - 9.0	20,000
B_6, g	200	3000
B_{12}, g	1.4	6×10^{-6}
Biotin, g	14	.0003
Pantothenic Acid, mg	4	10
Vitamin A, IU	500 - 8500	3750
Vitamin D, IU	100	200
Vitamin E, IU	1.4	20
Vitamin C, mg	146	100
Calcium, mg	2100	600
Phosphorus, mg	1000 - 1900	500
Potassium, mg	1000	1250
Sodium, mg	340	500
Magnesium, mg	200	300
Chloride, mg	1000	1000
Iron, mg	1.33	9
Zinc, mg	4.94	10
Manganese, mg		2.0
Copper, mg		1
Iodine, mg		.075
Energy, kcal	1030	1000

Development of Feeding Regimen

The elemental diets were fed by bottle to the first litter of piglets that received these formulas. Subsequent piglets fed these diets were fed via gastric catheters. The formulas were fed to the bottle-fed piglets at the rate of 15 ml/h on d 1 and 35 ml every 2 h on d 2. The rate was to be increased by 5 ml/feeding on each successive day, remaining at 50 ml/2h on d 6 and 7. Because the piglets in this litter suffered from severe, watery diarrhea, the formula was changed to Peptamen alone, at two-thirds strength on d 5. Subsequent litters of Group E piglets, fed via gastric infusion, received 10 ml $\cdot$ h^{-1} $\cdot$ kg BW^{-1} on d 1. On each successive day, the amount fed to healthy piglets was increased by 3 $\cdot$ h^{-1} $\cdot$ kg BW^{-1}. The flow rate was not increased for piglets that were not thriving. The last two litters were fed the MCT and LCT diets at half-strength. This decreased the production of watery feces.

With ketamine anesthesia, long post-surgery recovery times delayed oral feeding. These piglets were often slow to show normal sucking and swallowing behavior after being anesthetized. Placement of a gastric catheter during surgery allowed commencement of enteral feeding much sooner than was possible with bottle-feeding. Enteral nutrition was initiated on an individual basis when the piglet appeared to be awake and kicking motions of the hindlegs were observed. Usually, these animals remained on i.v.

fluids post-surgically for about 8 to 12 h before receiving enteral nutrition.

To determine the effect of PNICU conditions on piglets, the Group C controls were bottle-fed a milk-based formula designed to be similar to the composition of sow milk. To determine the effect of surgery on PNICU piglets, four animals (surgery controls) were fed the milk-based diet via gastric catheters using a continuous infusion pump. The use of infusion pumps reduced the variation in post-absorptive state that might occur when the animals are euthanized at different times.

In summary, a total of 13 Group E piglets were fed the elemental diets. Four piglets were bottle-fed, while the rest received enteral nutrition via gastric catheter. Two piglets were fed the base formula, Peptamen, with no additions, via gastric catheter, to determine if the additions of calcium and phosphorus were contributing to the diarrhea. Of the 16 piglets fed the milk-based control formula, 12 were bottle-fed and four underwent surgery for gastric catheter placement and were fed via constant infusion pump.

Daily Care

The piglets were monitored continuously throughout the trial. Urine was collected asceptically from the bladder catheters every 30 min. At morning and evening "rounds" the

incision site was checked and swabbed with an iodine solution, the venous catheter was flushed, bandages changed, and temperature, respiration, and girth measurements were recorded. When piglets were fed via gastric catheter, new bags of formula were started after rounds. Piglets were weighed during morning rounds. On d 3 and d 5 blood was collected from the umbilical catheter.

Every effort was made to keep stress on the piglets to a minimum. Each piglet was provided with a rubber baby-bottle nipple taped to the inside of the box, a plastic ball, and a soft cloth.

Tissue Collection

At sacrifice, the animals were anesthetized with Na pentobarbital and then exsanguinated by cardiac puncture. Organs were removed as quickly as possible, weighed, and frozen for later analyses. The intestines were measured, weighed, cleaned of contents, and then re-weighed. The small intestine of each piglet was divided into three segments of equal length, designated proximal (top) intestine (TI), middle intestine (MI) and distal (bottom) intestine (BI).

Analyses

Leukocyte (WBC) counts were performed using a Unopette dilution chamber and a hemocytometer. Microhematocrit (Hct) determinations performed shortly after blood was collected.

Hemoglobin (Hb) was determined using the modified Drabkins method with a Beckman DU-7 spectrophotometer at a wavelength of 540 nm.

The plasma glucose was assayed using Trinder reagent (Sigma Chemical Co., St. Louis), based on the glucose oxidase reaction. The OD was read with the Beckman DU-7 spectrophotometer at 505 nm.

Non-collagen protein in intestinal homogenates was determined using the micro-Biuret method (Itzhaki and Gill, 1964) after precipitation with .2 N NaOH. The OD was measured with a Gilford spectrophotometer at 310 nm.

The activities of the intestinal disaccharidases maltase, lactase, and sucrase were determined using a slightly modified technique by Dahlqvist (1964). Preparation of the tissue included homogenization in a glass-on-glass hand homogenizer with .05 M sodium phosphate buffer in a 1:5 weight/volume ratio. Each tissue homogenate was incubated with each substrate to determine the micromoles of glucose produced by the disaccharidase enzyme per microgram tissue, during a 1 h incubation. The OD due to the glucose produced was read at 505 nm with the Beckman DU-7 spectrophotometer.

Urine was collected from Group E piglets every 30 min throughout the trial. All of the urine collected during a 6 h period was pooled, unless it was unusual in appearance. In this case, the abnormal urine was placed in a separate vial. The urine was kept on ice or refrigerated until analysis.

The volume of urine collected as well as a description of its general appearance were recorded for each piglet for each 6 h period. Fresh urine was analyzed using Ames Multistix SG reagent strips (Miles, Inc., Ames). These strips provided a quick assessment of specific gravity, pH, protein, glucose, ketones, bilirubin, blood, and urobiligen. The remaining urine was frozen, pending further analysis.

The following equation was used to calculate the % BW gained by the 7-d old piglets:

% BW wt gain = ((d 7 BW - birth BW) ÷ birth BW) * 100.

The percent of body weight of each section of the small intestine (TI, MI, and BI) was calculated using the following equation: SI section wt(% of d 7 BW) = (SI section wt ÷ d 7 BW) * 100. These results will be referred to as %TIWT, %MIWT, and %BIWT.

Additional Information

Additional information and details of surgery, daily care, necropsy procedures, and tissue analyses are described by Baltzell (1988). The PhD dissertation involved the the

colostrum-deprived neonatal piglet model that was further developed in this current work.

Statisical Analysis

Treatment means for SF and B0 data only were obtained by method of least squares ANOVA (Snedecor and Cochran, 1969) using the GLM procedures of the SAS Statistical Software (Freund and Littell, 1981). The model included treatment, litter(treatment), sex, sex*treatment, and sex*litter(trt). Data from Group C and Group E treatments were not analyzed because of the low numbers of animals in each group.

Results

Numerical results are expressed as least squares means ± SE, unless stated to be observed means.

General Observations

Sow-fed piglets. All of the SF piglets were active, healthy and growing well at the end of the 7 d trial period. They did not seem unduly stressed by the daily weighing procedure.

PNICU piglets. Thirty-eight piglets were started on trial. All of the Group C piglets werew healthy and active through by d 7. Although the elemental diets were formulated to be similar to sow milk, the Group E piglets did not thrive. These piglets experienced diarrhea that may

were made for each litter. These changes reduced but did not eliminate the diarrhea. The survival rate of Group E piglets was low, as only nine of 20 survived for 7 days. This was probably due, in part to diet and to dislodged or faulty catheters. Piglets that had dislodged gastric or bladder catheters were humanely euthanized. Because of the low survival rate of the Group E piglets, only descriptive results will be presented.

Occasionally, piglets were born with umbilical hernias. This was not generally not a problem, except for one piglet, who diet after a portion of the small intestine strangulated. Blockages or strictures of the small intestine were observed in four animals.

Behavior of PNICU piglets. The hours of care required by the piglets allowed for close observation of their behavior. Each piglet had a particular activity pattern that was known to all the caretakers. Therefore, early detection of problems was possible.

The non-surgery piglets, bottle-fed the milk based diet, quickly became accustomed to the routine in the PNICU. They were noisy and wanted attention from the caretakers. Even during the first day, they began to recognize the pre-feeding routine. They had strong suckling reflexes. Play was part of their daily activity, just as it was for the sow-raised piglets. Although they were kept in individual boxes, the plexiglass allowed them to see each other and the

boxes, the plexiglass allowed them to see each other and the caretakers. Objects such as crumpled paper towels, plastic balls, and pacifiers were used as toys.

The healthy surgery piglets also exhibited play and attention-seeking behavior and did not seem hampered by the i.v. or enteral infusion lines attached to them. Soft cloths were used as bandages to cover the incision and keep lines in place. These were ignored by the animals. Swivels in the infusion lines kept the twisting and tangling to a minimum. These lines were changed twice daily, when fresh formula bags were set up.

Activity level (sleeping, lying down, alert, moving, etc.) of each piglet was recorded on the individual charts every half hour and changes from the previous record noted. Nasal oxygen was available, if needed. An attempt was made to cool (water or alcohol swabs) or warm (heating pad or heat lamp) animals whose body temperatures were not in the normal range.

Healthy animals generally produced an adequate amount of relatively clear urine. Animals that had decreased urine output or bloody urine generally had enlarged kidneys on necropsy and often had fluid accumulation in the abdomen and hindlegs.

In an effort to improve the survival and growth rate of the piglets fed the elemental diets, modifications in the formulas and daily intakes were made for each successive

litter. After the first litter, the calcium and phosphorus additions were discontinued. The amount of whey added to the base formulas was decreased. The formula strength and flow rate during the first few days after surgery were decreased. It was thought that these changes might decrease the diarrhea, improve tolerance of the formula, and smooth the transition to enteral nutrition after surgery.

Gross Observations at Necropsy

Sow-raised and B0 piglets. There were no abnormalities noted in the organs of any of the SF or the B0 piglets. The intestinal tissue of the B0 piglets was very thin compared with that of the SF and could be easily torn when separating the mesentery from the small intestine.

The stomachs of the B0 piglets essentially were empty, while the stomachs of SF piglets contained milk curd. The proximal intestinal contents of the SF piglets were very liquid and yellowish. The contents became thicker in the more distal areas. The cecal contents were pasty and darker yellow. Large intestine contents were very thick and became darker in the more distal regions. The distal colon contained formed fecal pellets, dark in color.

Post-surgical, formula-fed piglets. In all cases, the positions of the catheters were verified on necropsy. No abnormalities, other than occasional adhesions, were noted

with the catheters. The lining of the stomachs and bladders did not show irritation from the catheters.

Some piglets exhibited respiratory distress and had reddish, congested lungs at necropsy. Some of these animals had displayed swollen shoulders, hindlegs, and abdomens with bluish blotches seen beneath the skin. While cause of these blotches is unknown, it may have been due to caretakers holding the piglets too tightly during rounds.

Many animals with bladder catheters had enlarged kidneys. In some cases, the ureters also were enlarged.

The livers of all of the piglets appeared normal, but several piglets had gall bladders that contained a dark, thick material. This was not seen in the SF or BO groups.

Non-surgical, formula-fed piglets. The Group C piglets, fed the conventional, milk-based diet, were healthy and active at the end of the 7 d. The organs of these piglets appeared normal at necropsy.

Some piglets bottle-fed the elemental formulas had slightly reddish, congested lungs.

Weight Gain

The means reported in this section are observed means ± SE.

Sow-fed litters. The sow-fed piglets (SF) increased their birth weight by 65.75%. One litter (n=13) gained an average of 74.49% ± 4.24, with a minimum of 50.76% and a

maximum of 115.18%. The other litter (n=9) gained an average of 53.13% ± 5.90. The least amount gained was 27.58%, and the most 79.16%.

Formula-fed piglets. The birth weight of Group C piglets (n=12) increased by an average of 78.56% during the week long trial. The smallest gain was 54.40%, while the largest was 96.41%. The surviving surgery controls, fed the milk-based diet via gastric catheters (n=2), gained only 29.75%.

One piglet fed one of the MCT-based diets gained 5.63% of its birth weight and one piglet increased its weight by 21.91%. The other piglets experienced losses of 6.89%, 11.09%, and 8.5%. The MCT-fed piglets were all very thin by the end of 7 d. None of the four surviving piglets fed one of LCT-based lost weight, but they gained very little. The average gain was 11.99%. The two bottle-fed piglets gained 6.65% and 1.85% of their birth weight. The other two, fed by gastric infusion, gained 35.29% and 4.18%. All but two Group E piglets weighed greater than 1 kg at birth, and these exceptions were over 900 g.

Small Intestine Length

The total length of the small intestine (SI) was measured at necropsy. The length of the SI in the B0 piglets was used as a reference baseline value.

The least squares means (LS means) for one litter of B0 and one litter of SF piglets were 284.39 cm ± 11.27 and 371.73 cm ± 9.34, respectively.

Blood Glucose

Blood glucose may be expected to vary depending on an animal's nutritional status, health, and physical condition. The use of infusion pumps may have reduced variations due to differences in times after feeding at death. This was difficult to control with bottle feedings due to time and labor constraints. The results in this section are reported as observed means unless stated otherwise.

The LS means of blood glucose concentration of two litters of SF piglets and one litter of B0 piglets were 13.37 mM ± 1.81 and 6.55 mM ± 2.03, respectively.

Small Intestine Weight

The %TIWT, %MIWT, %BIWT observed for B0 piglets, and sow-fed piglets are presented in Table 2.3. The B0 mean was obtained from data from one litter and the SF mean was obtained from two litters.

Disaccharidases in the Small Intestine

Results of the disaccharidase analyses are calculated based on the amount of glucose produced by 1 g of tissue as a result of substrate hydrolysis occurring during a 1 h

incubation period. The means for the enzyme activities for SF and B0 piglets are presented in Tables 2.4-2.6. The results for the SF and B0 piglets are from one litter of each group. Therefore, the standard errors in the tables also include litter effects.

Group C piglets (n=12), fed a milk-based formula, and the Group E piglets had very low intestinal sucrase activities. In tissue samples from several Group E piglets no sucrase activity was detected.

Maltase activity was detected in Group E piglet intestines, as well as in tissue obtained from Group C piglets even though the Group C piglets had not ingested maltose. Similarly, lactase activity was detected in the Group E piglets that had never ingested lactose.

Table 2.3. Least squares means for the proximal, middle, and distal sections of the SI, expressed as a proportion of body weight.

Treatment	%TIWT	SE	%MIWT	SE	%BIWT	SE
Sow-fed (SF)	.83	.03	.84	.03	.87	.03
Birth (B0)	.58	.04	.63	.03	.62	.04

Table 2.4. Least squares means of sucrase activity in proximal, middle, and distal sections of the small intestine of 7-day old sow-fed piglets and piglets killed at birth.

	Sucrase Activity μmol glucose produced/h/g wet tissue					
	TI		MI		BI	
Treatment	Mean	SE	Mean	SE	Mean	SE
B0	1.2	.85	<.1	.94	1.2	.70
SF	6.6	1.18	3.2	1.31	.6	.98

Table 2.5. Least squares means of maltase activity in proximal, middle, and distal sections of the small intestine of 7-day old sow-fed piglets and piglets killed at birth.

	Maltase Activity μmol glucose produced/h/g wet tissue					
	TI		MI		BI	
Treatment	Mean	SE	Mean	SE	Mean	SE
BO	14.1	3.84	14.2	3.74	11.7	.93
SF	44.2	4.22	46.7	5.19	23.2	1.29

Table 2.6. Least squares means of lactase activity in proximal, middle, and distal sections of the small intestine of 7-day old sow-fed piglets and piglets killed at birth.

	Lactase Activity μmol glucose produced/hour/gram wet tissue					
	TI		MI		BI	
Treatment	Mean	SE	Mean	SE	Mean	SE
B0	45.1	8.89	217.2	24.23	82.3	8.07
SF	34.4	12.35	171.3	33.65	74.4	11.21

Complete Blood Counts

The complete blood counts (CBC), consisting of Hb, Hct, and white blood cell counts, were performed to determine if the blood components measured fell within published normal ranges for piglets of this age. The CBCs provided a method of monitoring the general health of the piglet during and after 7 d on the trial. However, it was difficult to collect blood from the umbilical artery catheter on d 3 and 5 of the trial as the catheters often became blocked and only a very small amount of blood, if any, could be withdrawn. Not enough data was collected on these days to provide meaningful results. Data from SF and B0 piglets is presented in Table 2.7.

Table 2.7. Results of CBCs from blood collected at necropsy via cardiac puncture from sow-fed piglets and piglets killed at birth.

Treatment	n[a]	Hb (g/dl)	Hct (%)	WBC (cells/ml^3)
SF	22	5.6-10.5	18.7-35.3	7000-18,700
BO	9	3.3-20.5	10.3-42.2	4050-10,100
7-d sow-fed[b]	31	3.6-5.2	24.1-34.9	8900-12,700[c]
1-d sow-fed[b]	70	12-12.7	39.6-43.5	6270-17,600

[a]number of samples obtained

[b]Schmidt and Tumbleson, 1986

[c]d 28 data

Urinalysis

After surgery to introduce a bladder catheter, the piglets routinely had some amount of blood in the urine. Piglets with low urine outputs tended to have urine than was visibly bloody, while piglets with substantial urine output tended to have clear, yellow urine that was found to contain blood only by chemstrip analysis.

The urine routinely contained varying amounts of protein. This also may be expected after surgery. The pH of the urine varied among piglets, but an individual piglet tended to have urine in a small pH range throughout the trial. The apparent health of the piglets did not seem related to the changes in urine pH or specific gravity.

Discussion

The purpose of the work using piglets was to learn to develop techniques and procedures to be used in a trial designed to evaluate an elemental, enteral diet for sick or premature neonates. To achieve this, two elemental formulas and one milk-based formula were fed to the neonatal colostrum-deprived piglets for 7 d. The elemental formulas differed in the amount of fat provided by LCT and MCT lipids. The elemental diets contained malto-dextrin as the carbohydrate source, rather than lactose because compromised neonates may have reduced lactase activity.

The animals in the first litter were bottle-fed the elemental diets. None of them appeared to thrive during the 7 d study period, probably due to diarrhea and respiratory problems. All subsequent Group E piglets were fed via gastric catheters.

The diets were modified for each litter in an attempt to develop a formulation suitable for the piglets. Changes in the diet and flow rate reduced but did not eliminate the diarrhea. Some workers had suggested that maltase may be low in neonatal piglets. Therefore, it was likely that undigested maltose reaching the large intestine may have caused the diarrhea. This led to the investigation of the carbohydrate source, maltose.

Disaccharidases in Neonatal Piglets

Human infants have adequate maltase at birth to digest maltose even when they are born prematurely (Paige and Bayless, 1981). However, there are conflicting reports in the literature as to when maltase becomes active in the newborn piglet. Very low maltase activity activities have been detected in the small intestine of piglets as early as 2 d of age (Dahqvist, 1961a), and the activity appears to increase from d 3 to 10 (James et al., 1987). The maltose fed to the Group E piglets was within the amount reported to be digested by normal, sow-fed piglets, but it is possible

that these colostrum-deprived piglets lacked sufficient total tract maltase activity to meet their energy needs on a maltose-containing diet.

Investigation of the tissue disaccharidase activities in the small intestine of the experimental piglets was undertaken to determine the ability of newborn and 7-d old piglets to digest sucrose, maltose, and lactose.

Lactase activity was measured for reference as this enzyme should be very active during the first week after birth. Sucrase also would be a useful reference because only low activities have been reported in 1-wk old piglets (Veum and Mateo, 1986).

The large variation in disaccharidase activities between piglets in this trial was also reported by othe workers (Manners and Stevens, 1972). It is difficult to interpret the data when the large variation is coupled with low numbers of animals per treatment.

The lactase activity along the SI was similar for the B0 and SF piglets. This is to be expected, because mammals must be prepared, at birth, to digest lactose. The Group E piglets, although not thriving, did have lactase activity, even though they never consumed lactose. The intestine remained prepared for the ingestion of this sugar throughout the week.

The B0 piglets had low maltase activities, leading to the conclusion that piglets may not be able to digest

maltose efficiently enough to meet their energy needs when this sugar is the sole carbohydrate source. The sow-fed piglets appeared to have a numerically higher maltase activity than did the piglets killed at birth even though the SF piglets had never ingested maltose. This suggested that age is a factor in enzyme development. Birth and feeding, especially of colostrum, have been shown to stimulate enzyme and intestinal development (Widdowson, 1985).

Sucrase activities were very low, compared with the other disaccharidases measured. Dahlqvist (1961a) found no appreciable sucrase in 2-d old piglets, but Bellis (1957) reported equal digestion of lactose and sucrose by d 3. Manners and Stevens (1972) were barely able to detect sucrase activity until the piglets were 1 wk old. James et al. (1987) found that EGF increased sucrose in neonatal piglets. EGF is secreted in colostrum. In the present study, only the SF ingested colostrum and this may explain the generally higher enzyme activities measured in this group.

Growth Rate in Neonatal Piglets

The Group C controls grew at a rate comparable with, that of the sow-fed piglets. While the hand-raised piglets did not have to expend energy to maintain body temperature or compete for food, they did not have the benefit of

colostrum or sow's milk. Even though the growth rate of Group E piglets was poor, the growth rate of the Group C piglets indicated that colostrum-deprived piglets can be raised successfully raised for 7 d in an intensive care facility. The unit used in this study was kept extremely clean but not sterile.

Development of the Small Intestine in Neonatal Piglets

It was suggested that the ingestion of colostrum and milk affect the growth and development of the small intestine in newborn mammals (Widdowson, 1985). An ideal, enteral formula should supply adequate nutrients and growth factors, especially to the small intestinal tissue. The piglets killed at birth had very small, fragile intestines that could be distinguished easily from the intestines of older, fed piglets. By 1 wk of age, the small intestines of SF piglets were longer and heavier than the B0 intestines. Because these weights are on a wet tissue basis, it is not known whether the heavier weights are due to fluid or dry matter.

Blood glucose

Although blood glucose is regulated by insulin and glucagon, it can be affected by nutritional state, general health, and time after feeding. Piglets are born with low energy stores that must be replenished shortly after birth.

Blood glucose was low in B0 piglets. These piglets often were not sacrificed for several hours after birth, so partial depletion of their energy stores probably occurred.

Normal blood glucose for neonatal piglets has been reported by Tumbleson and Schmidt (1986). The values had large standard errors, which is expected as glucose varies with time after feeding. They reported mean values for birth and 1-wk old piglets of 2.67 ± 1.06 and 6.67 ± 1.11 mM/L, respectively. The birth value is lower than the 6.63 ± 1.54 mM measured in piglets killed shortly after birth in this study. All of the Group E piglets had blood glucose concentrations similar to the reported values for 1-wk old piglets. However, the 1-wk old SF and Group C piglets had concentrations averaging about 13 mM. Method of blood collection, stress, nutrition, breed effects, and time of sampling have been known to affect results (Tumbleson and Schmidt, 1986).

Hematology

Results of the CBCs were compared with values reported by Schmidt (1986). Hemoglobin in neonatal piglets was reported to be 12.5 ± .2 g/dl at birth, and 10.0 ± 1.8 g/dl by d 7. The Hct values at birth are 40.3 ± .7% and 29.5 ± .4% by d 7. The piglets in the present study had considerable variation in the Hb and Hct values, but most were within or close to the reported ranges. However, the

PNICU surgery controls (n=2) had low Hb and Hct values. These piglets had been very weak and may have had problems unrelated to the treatment or surgery.

At birth, piglets would be expected to have about 6000-1700 WBC/μl, and 7000-10,000 WBC/μl by d 28 (Schmidt, 1986). Generally, the piglets that survived until d 7 had CBC values that were close to the normals for their age. Many of the Group E piglets had severe diarrhea but did not have extremely high packed cell volumes, suggesting that the formula flow rate was sufficient to prevent dehydration.

Summary of Piglet Trial

As a result of this study, some techniques and procedures for performing trials on colostrum-deprived piglets were refined. Although each successive study will probably result in further improvements, the colostrum-deprived piglet model currently can be used successfully to investigate the nutritional needs of neonates.

The poor growth rates of the piglets fed the MCT and LCT diets may have been due to the carbohydrate source. No information regarding the benefits of the addition of MCT to neonatal formulas could be obtained from this trial. However, using an appropriate base formula should allow this information to be obtained from future studies using the piglet model.

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It is clear that only digestible carbohydrates should be included in neonatal enteral formulas. However, information on the digestibilities of various sugars may not be available for each species. Development of an enteral diet for foals based on piglet data was a goal of this research. However, it was not known whether newborn foals had sufficient maltase activity to adequately digest the malto-dextrins in the base elemental diet. Therefore, an oral disaccharide tolerance test was performed on neonatal foals.

CHAPTER III
ORAL DISACCHARIDE TOLERANCE TEST FOR NEONATAL FOALS

Introduction

Neonates that are sick, stressed, or born prematurely may have poorly functioning digestive systems. The pattern of small intestine disaccharidase development may not be the same for these newborns as for healthy neonates (Rossi et al., 1986). Lactase activity can be reduced greatly as a result of prematurity or disease (Rossi et al., 1986; Tzipori et al., 1984). Consequently, lactose may not be suitable for use in enteral diets designed to support non-healthy neonates. The use of other carbohydrate sources needs to be considered carefully. Including monosaccharides in enteral diets would increase the osmolarity of the formula higher than neonatal intestines could handle well, possibly causing diarrhea. Complex sugars must be cleaved into smaller and smaller units, and finally from disaccharides into single sugar molecules before absorption can take place. It is not known if neonatal foals can digest and absorb disaccharide sugars other than lactose. Therefore, it is important to determine which enzymes are likely to be active in the intestine of equine neonates.

Enzyme activity varies with age and species and can vary among similar individuals (Paige and Bayless, 1981). There is very little information in the literature about the disaccharidase activity in the small intestine of foals, although it was studied in neonatal humans, pigs, calves, and rats (Rossi et al., 1986; Dollar and Porter, 1957; Code, 1968; Paige and Bayless, 1981).

Dollar and Porter (1957) concluded from their study of newborn calves that enzyme activities determined from mucosal cell extracts were correlated closely to the results of an oral disaccharide tolerance test. Using a similar procedure, Roberts et al. (1974) studied lactase, maltase, and sucrase activities in horses of various ages by homoginizing samples of intestinal mucosa. Small intestine tissue was obtained from the horses slaughtered or euthanized. However, the data does not show the pattern of disaccharidase development during the critical first week postpartum. Lactase was detected as early as 105 d gestation. Peak activity was found in foals at birth and it remained high for 4 mo. Sucrase was barely detected in fetuses from 3 to 9 mo gestation, remaining low at birth. Adult activities were attained by 7 mo of age. Similarly, maltase was very low until the ninth month of gestation. Activities at birth were 12 to 15% of the enzyme activities of adult equines. The authors reported a gradual increase, until adult activities were reached at 7 mo of age.

In a subsequent study, Roberts (1975) determined the ability of horses to digest and absorb disaccharides using an oral disaccharide tolerance test. These results agreed with those of his earlier study. However, this study did not include data on very early neonatal life.

A non-invasive method of estimating disaccharidase activity, such as an oral tolerance test, would allow foals to be studied at more than one point in time and would not require the experimental animals to be euthanized.

The present study was done using healthy foals, nursing their mothers. Although the results will not necessarily be applicable to sick or premature foals, it may eliminate some disaccharides as possible ingredients for enteral diets; if healthy foals are unable to digest a particular disaccharide, then it is unlikely that a stressed foal would have that ability. The results of this study will provide information on the digestive ability of newborn, healthy foals non-invasively, and help determine possible sources of carbohydrates for use in enteral formulas. Because the animals do not need to be euthanized to obtain samples, each foal can be used on different days to determine the changes in enzyme activity during the first week of life. There can be considerable variation in activities among animals (Manners and Stevens, 1972).

Selection of a carbohydrate source for inclusion in elemental, enteral formulas will depend on the activity of

the necessary enzymes and the desired osmolarity of the formula. Disaccharidases are important in the digestion of all sugars larger than monosaccharides.

It was presumed that healthy foals would have adequate lactase activity while relying on milk for nourishment. It was also presumed that glucose would be easily absorbed by these neonates. Therefore, the changes in plasma glucose occurring after administration of glucose and lactose would be used for comparison with test sugars. Maltose and sucrose were chosen as test sugars. Maltose was included because it was the carbohydrate source in the base formula fed to the piglets. Sucrose was tested because it was thought that this sugar would be poorly digested and would provide a reference for a non-absorbed substrate.

The goals of this trial were:

1. to investigate the ability of newborn, healthy foals to digest maltose, lactose, and sucrose during the first 5 d postpartum;

2. to investigate the change in blood glucose in healthy foals after a short fast and the effects of age on the ability of the foals to maintain blood gluocse concentration after a short fast; and,

3. to determine the changes in blood glucose in healthy foals in response to an oral glucose challenge.

Materials and Methods

Preliminary Study

A preliminary study to determine the effect of nursing on blood glucose was performed on a normal, healthy foal at 8 h postpartum. Care of the mare and foal during and after birth followed standard procedures at the University of Florida Horse Research Center.

At 8 h of age, the foal was fitted with a Teflon jugular vein catheter (Quik-Cath, Travenol Laboratories, Inc.), in an aseptic procedure, to allow periodic blood sampling. To minimize stress to the foal, a small amount of local anesthetic was injected at the catheter site. The plastic wings of the catheter were fixed to the skin with a superglue adhesive. This was easily removed with acetone at the conclusion of each trial period. The catheter was filled with sterile, heparinized saline between samplings. The foal was then allowed to return to the mare and resume normal activity. After the foal nursed the mare, the time was noted and the foal was muzzled to prevent nursing. Blood was drawn from the catheter at this time (time = 0). Blood was then drawn every 30 min thereafter for 2 h. This procedure was repeated on the same foal on d 5. This study suggested that blood glucose reached a baseline concentration after a 1 h fast. It was concluded that a 2 h fast prior to administration of the treatment, and a 4 h fasting collection period would be sufficient to monitor

changes in blood glucose after a test meal in the subsequent experiment.

Animals and Design

Eleven Quarter Horse and two Thoroughbred foals were used for the main trial. The foals remained with their dams throughout the trial. Foals were weighed shortly after birth and on d 5. After each foal was born, it was randomly assigned, by sex, to one of four treatment groups until the block was filled. This procedure was repeated for subsequent foals, assigning foals of each sex to fill the treatment blocks evenly.

On d 1, at 6 h postpartum, each foal was fitted with a jugular vein catheter, as described above. Foals were muzzled to prevent nursing. The foals were then fasted for 2 h to allow blood glucose concentrations to reach a baseline level. Each animal was then given the appropriate treatment consisting of oral administration of either maltose, lactose, or sucrose at 1 g (5.56 mmol) per kg BW in a 20% solution (w/v), or a glucose solution at .5g (2.78 mmol) per kg BW. These dosages were similar to dosages used by Roberts (1975).

The solution was offered to the foal in a bottle fitted with a suitable nipple. If the foal did not voluntarily consume the solution, it was administered via dosing syringe

or via nasogastric tube. Blood was collected from the catheter immediately before the solution was given (time = 0), every 15 min for the first hour, and every 30 min thereafter for the second, third, and fourth h of the trial period. Each foal received the same treatment when the procedure was repeated on d 3 and d 5 postpartum.

Analyses

The blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA), plasma removed after centrifugation at 3500 *g* for 20 min and frozen. Plasma glucose was determined using the Trinder peroxidase method (Sigma Chemical Co.). Optical density was measured at 505 nm with a Beckman DU-20 spectrophotometer.

Statistical Analyses

Statistical analyses were performed by method of least squares ANOVA with time as a class variable (Snedecor and Cochran, 1969,) using the GLM procedures of SAS (Freund and Littell, 1981). The complete mathematical model is given in Table 3.1. The appropriate error term is shown for each independent variable.

Table 3.1. Complete mathematical model for ANOVA of changes in plasma glucose.

Source	df	Error term
Treatment	3	Foal(Treatment*Sex)
Sex	1	Foal(Treatment*Sex)
Treatment*Sex	3	Foal(Treatment*Sex)
Foal(Treatment*Sex)	5	Remainder
Day	2	Day*Foal(Treatment*Sex)
Treatment*Day	6	Day*Foal(Treatment*Sex)
Day*Sex	2	Day*Foal(Treatment*Sex)
Treatment*Sex*Day	6	Day*Foal(Treatment*Sex)
Day*Foal(Treatment*Sex)	10	Remainder
Time	10	Time*Foal(Treatment*Sex)
Time*Treatment	30	Time*Foal(Treatment*Sex)
Time*Foal(Treatment*Sex)	50	Remainder
Day*Time	20	Remainder
Sex*Time	10	Remainder
Day*Sex*Time	20	Remainder
Treatment*Sex*Time	30	Remainder
Day*Treatment*Time	60	Remainder
Treatment*Day*Sex*Time	60	Remainder
Remainder	101	

Equations for the time curves were obtained using reduced models with time as a continuous variable. The reduced pooled model is presented in Table 3.2. The models for comparison of day curves, treatment curves and day*treatment curves were similar to the pooled model except that day*time^n, treatment*time^n, and day*treatment*time^n, respectively, were used in place of time^n. F tests for homogeneity of the time curves were calculated based on the techniques described by Snedecor (1956). Rejection of the null hypothesis would indicate that the curves differed, i.e. were not parallel.

Table 3.2. Pooled regression model[a] for plasma glucose time curves.

Pooled Curve

Source	df	Error term
Treatment	3	Foal(Treatment*Sex)
Sex	1	Foal(Treatment*Sex)
Treatment*Sex	3	Foal(Treatment*Sex)
Foal(Treatment*Sex)	5	Remainder
Day	2	Day*Foal(Treatment*Sex)
Treatment*Day	6	Day*Foal(Treament*Sex)
Treament*Day*Sex	6	Day*Foal(Treatment*Sex)
Day*Sex	2	Day*Foal(Treatment*Sex)
Day*Foal(Treatment*Sex)	10	Remainder
Time	1	Remainder
$Time^2$	1	Remainder
$Time^3$	1	Remainder
$Time^4$	1	Remainder
$Time^5$	1	Remainder
$Time^6$	1	Remainder
Remainder	383	

[a]Reduced model

Results

General Observations

In general, the foals drank the treatment solutions readily from a bottle on d 1 of the trial. On d 3 and 5, some foals refused the solution. Some of these foals also proved uncooperative in swallowing aliquots of the solution delivered by dosing syringe. In these cases, a very small diameter nasogastric tube was passed, usually without unduly stressing the foal.

Some foals were uncooperative during catheterization. These foals were allowed to relax for periods up to 1 h after catheterization before the treatment was administered. Throughout the trial every effort was made to keep the foals calm. Subdued lighting, minimal restraint and keeping the mare nearby helped reduce the foal's anxiety.

None of the foals experienced any digestive disturbances, such as colic or diarrhea, during the trial. The foals became adjusted to the frequent blood collections, remaining in a recumbent position or even sleeping.

High quality reagent grade substrates were used for the treatment solutions. Analysis of the treatment solutions showed maltose contained 1.48% free glucose, sucrose contained .74%, and lactose less than .01%.

Fasting Blood Glucose

The plasma samples collected from all foals at time = 0 were used to determine fasting glucose concentrations. At time = 0 no treatment had been given to the foals and they had been treated alike. Therefore, data from all foals were pooled.

Breed and breed interaction effects were found to be non-significant ($P > .05$). Rogers et al. (1983) reported higher blood glucose concentrations in young fillies than in colts, although results of statistical analyses were not reported. However, we found no differences due to sex in the 13 foals studied ($P = .8957$).

The LS mean of blood glucose from fasted foals was 4.14 mM on d 1. The means on d 3 and d 5 were 6.08 and 6.31 mM, respectively. The SE of the means is .27. The mean from d 1 is lower than the mean from the other 2 d ($P = .0001$). Day 3 and d 5 means were not different from each other.

Glucose Absorption Time Curves

Using time as a continuous variable, equations for treatment, day, and treatment-day curves were calculated. Sequential addition of higher order terms showed time to the fifth power to be significant ($P < .05$). However, fifth order equations led to negative estimates near the right hand portion of some curves. Equations of the sixth order were then used, as these produced logical curves. Sixth

order equations were used to describe all of the curves. However, sequential addition of higher order terms revealed that the linear term only was sufficient to describe the plasma glucose time curves resulting from sucrose administration.

The equations for the day-treatment curves are presented in Tables 3.3-3.6. Time curve equations represent the changes in plasma glucose due to treatment administration. Thus, changes in plasma glucose occurring after dosing with oral glucose are represented by the glucose (G) time curves. Similarly, lactose (L), maltose (M) and sucrose (S) curves represent changes in plasma glucose in foals dosed with these sugars.

Treatment Curves Pooled Over All Days

Orthogonal contrasts and tests for homogeneity of the curves were performed on data from each treatment pooled over all 3 d. The L curve was not different from the M and S curves combined. The G curve was not different from the pooled M, L, and G curve. The M curve was different from (i.e. not parallel to) the S curve ($P = .0220$). The G and L curves also were different from each other ($P = .0186$).

Table 3.3 Equations for day*treatment curves for lactose.

DAY 1

$$Y^{a} = 5.224 + .0103T^{b} + 2.273x10^{-3}T^{2} - 3.979x10^{-5}T^{3} + 2.526X10^{-7}T^{4} - 7.105x10^{-9}T^{5} + 7.564x10^{-13}T^{6}$$

DAY 3

$$Y = 7.013 + .162T - 3.167x10^{-3}T^{2} + 1.632x10^{-5}T^{3} + 3.606x10^{-8}T^{4} - 4.982x10^{-10}T^{5} + 1.020x10^{-12}T^{6}$$

DAY 5

$$Y = 6.847 + .159T - 3.036x10^{-3}T^{2} + 1.805x10^{-5}T^{3} + 2.533x10^{-8}T^{4} - 8.952x10-11T^{5} + 2.195x10^{-13}T^{6}$$

[a] Y = plasma glucose, mM

[b] T = time (continuous variable)

Table 3.4 Equations for day*treatment curves for maltose.

DAY 1

$$Y^{a} = 5.361 + .00136T^{b} + 1.404x10^{-3}T^{2} - 2.634x10^{-5}T^{3} + 1.945X10^{-7}T^{4} - 6.494x10^{-10}T^{5} + 8.180x10^{-13}T^{6}$$

DAY 3

$$Y = 6.192 + .076T - 1.939x10^{-3}T^{2} + 1.806x10^{-5}T^{3} - 7.001x10^{-8}T^{4} + 9.031x10^{-11}T^{5} + 2.322x10^{-13}T^{6}$$

DAY 5

$$Y = 6.031 + .124T - 3.000x10^{-3}T^{2} + 2.885x10^{-5}T^{3} - 1.381x10^{-7}T^{4} + 3.145x10-10T^{5} - 2.535x10^{-13}T^{6}$$

[a] Y = plasma glucose, mM

[b] T = time (continuous variable)

Table 3.5 Equations for day*treatment curves for glucose.

DAY 1

$$Y^{a} = 3.020 + .167T^{b} - 2.041\times10^{-3}T^{2} - 1.381\times10^{-5}T^{3} + 3.102\times10^{-7}T^{4} - 1.558\times10^{-9}T^{5} + 2.510\times10^{-12}T^{6}$$

DAY 3

$$Y = 5.762 + .228T - .0114T^{2} + 1.981\times10^{-4}T^{3} - 1.553\times10^{-6}T^{4} + 5.615\times10^{-9}T^{5} - 7.623\times10^{-12}T^{6}$$

DAY 5

$$Y = 6.260 + .133T - 5.161\times10^{-3}T^{2} + 6.919\times10^{-5}T^{3} - 4.406\times10^{-7}T^{4} + 1.356\times10-9T^{5} - 1.622\times10^{-12}T^{6}$$

[a]Y = plasma glucose, mM

[b]T = time (continuous variable)

Table 3.6 Equations for day*treatment curves for sucrose.

DAY 1

$$Y^{a} = 3.692 - .120T^{b} + 3.408\times10^{-3}T^{2} - 4.227\times10^{-5}T^{3} + 2.506\times10^{-7}T^{4} - 7.007\times10^{-10}T^{5} + 7.408\times10^{-13}T^{6}$$

DAY 3

$$Y = 5.480 - .001T + 8.630\times10^{-4}T^{2} - 2.559\times10^{-5}T^{3} + 2.581\times10^{-7}T^{4} - 1.085\times10^{-9}T^{5} + 1.628\times10^{-12}T^{6}$$

DAY 5

$$Y = 6.748 + .035T - 1.299\times10^{-3}T^{2} + 1.721\times10^{-5}T^{3} - 1.110\times10^{-7}T^{4} + 3.410\times10\text{-}10T^{5} - 3.946\times10^{-13}T^{6}$$

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[a] Y = plasma glucose, mM

[b] T = time (continuous variable)

Comparison of Day-Treatment Curves

The least squares means for each treatment for each of the days are presented in Table 3.7.

Day 1. The M curve was different from the S curve (P = .0059), the L curve (P = .0015), and the G curve (P = .00002). The G curve was different from the L curve (P = .0028) (Figures 3.1-3.2).

Day 3. The M curve was not parallel to the L curve (P = .0027), but was not different from the G curve on this day (Figures 3.3-3.4).

Day 5. By d 5, there was no difference between M and L or between the M and G time curves. The G and L curves were not different when the foals were 5 d old (Figures 3.4-3.5). The S curve also was not different from the other three curves.

Table 3.7. Least squares means for each treatment and each treatment-day in mmol/liter of plasma glucose.

Treatment	Day 1	Day 3	Day 5	Mean	SE
Glucose	3.98	5.90	5.78	5.22	.25
Lactose	6.44	7.59	7.51	7.18	.25
Maltose	4.57	6.62	6.36	5.85	.23
Sucrose	3.44	5.83	6.29	5.19	.25

Least squares means were determined using a reduced model.

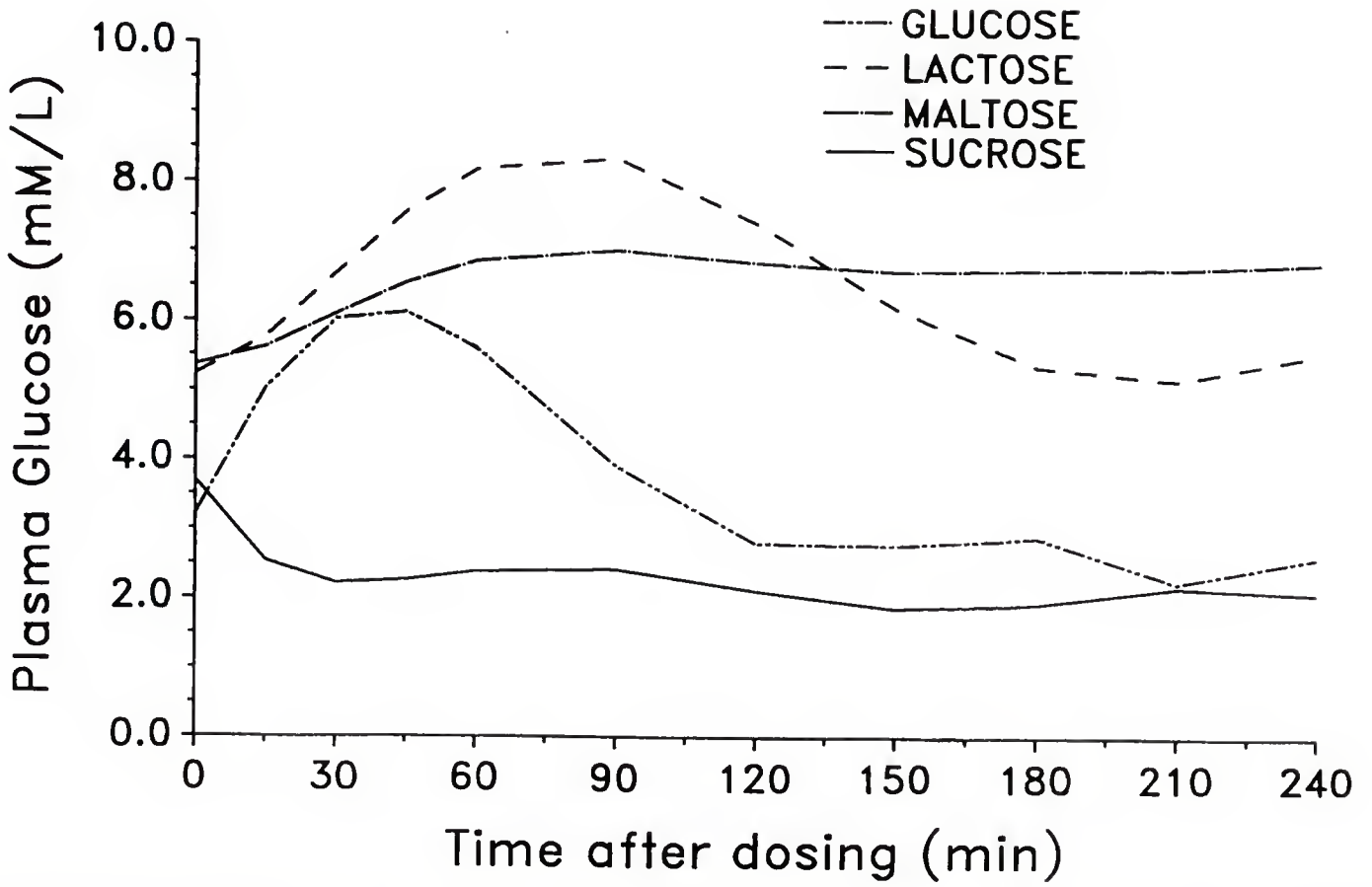

Figure 3.1. Changes in plasma glucose over time in 1-day old foals fed either glucose (SE mean = .15), lactose (SE mean = .18), maltose (SE mean = .12), or sucrose (SE mean = .17)

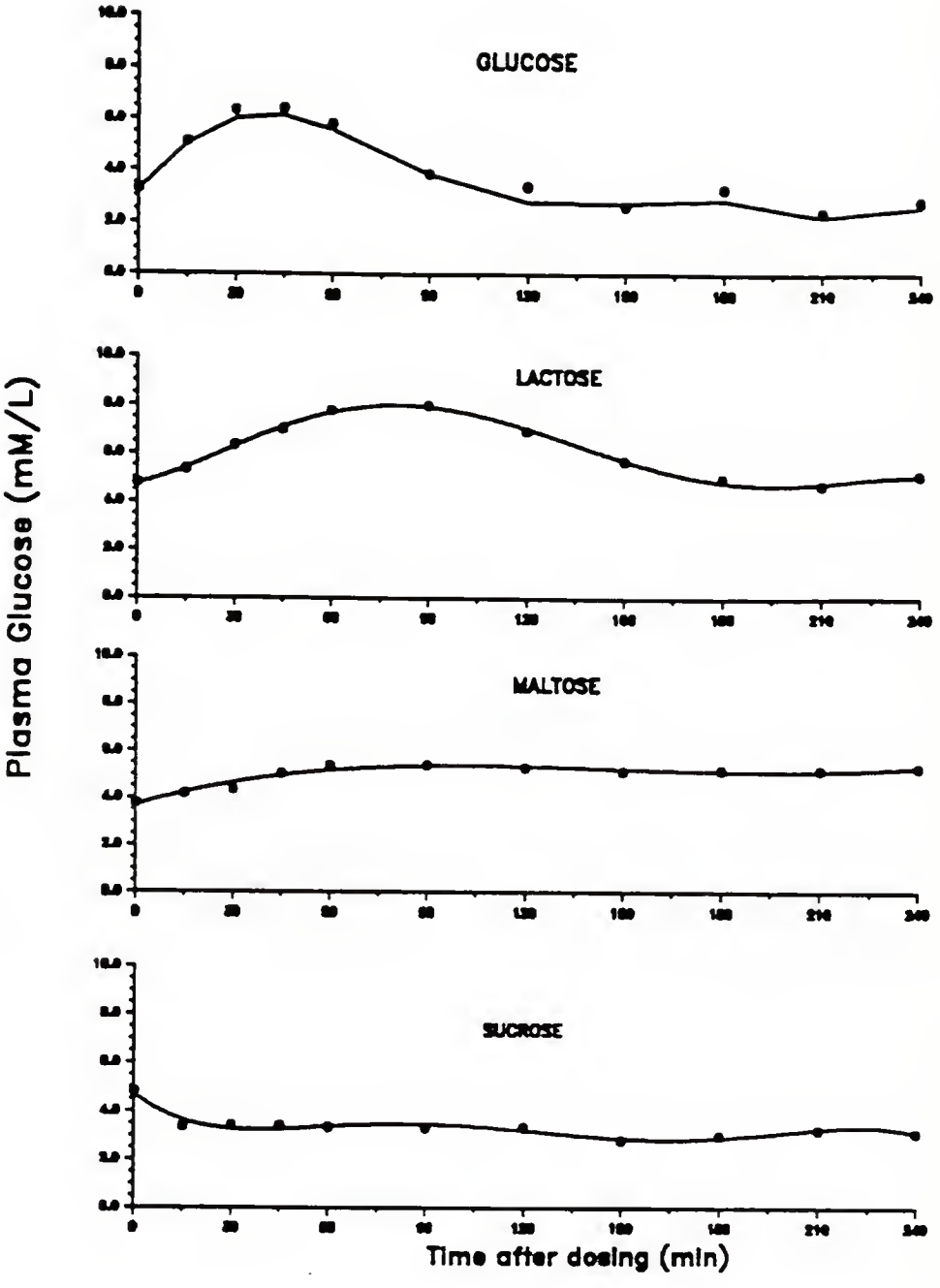

Figure 3.2. Changes in plasma glucose (least squares means) over time in 1-day old foals fed either glucose, maltose, lactose, or sucrose.

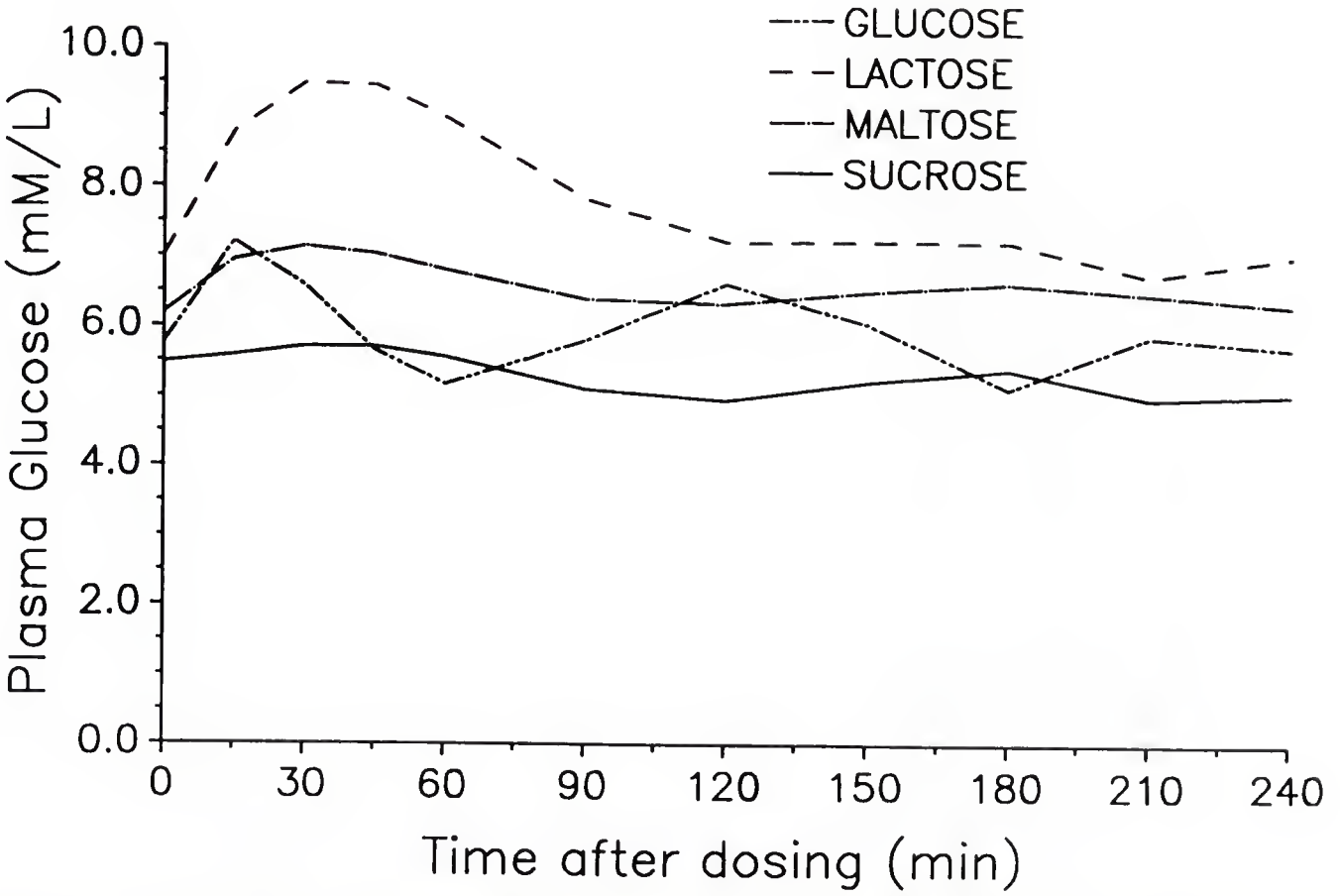

Figure 3.3, Changes in plasma glucose over time in 3-day old foals fed either glucose (SE mean = .26), lactose (SE mean = .12), maltose (SE mean = .08), or sucrose (SE mean = .08)

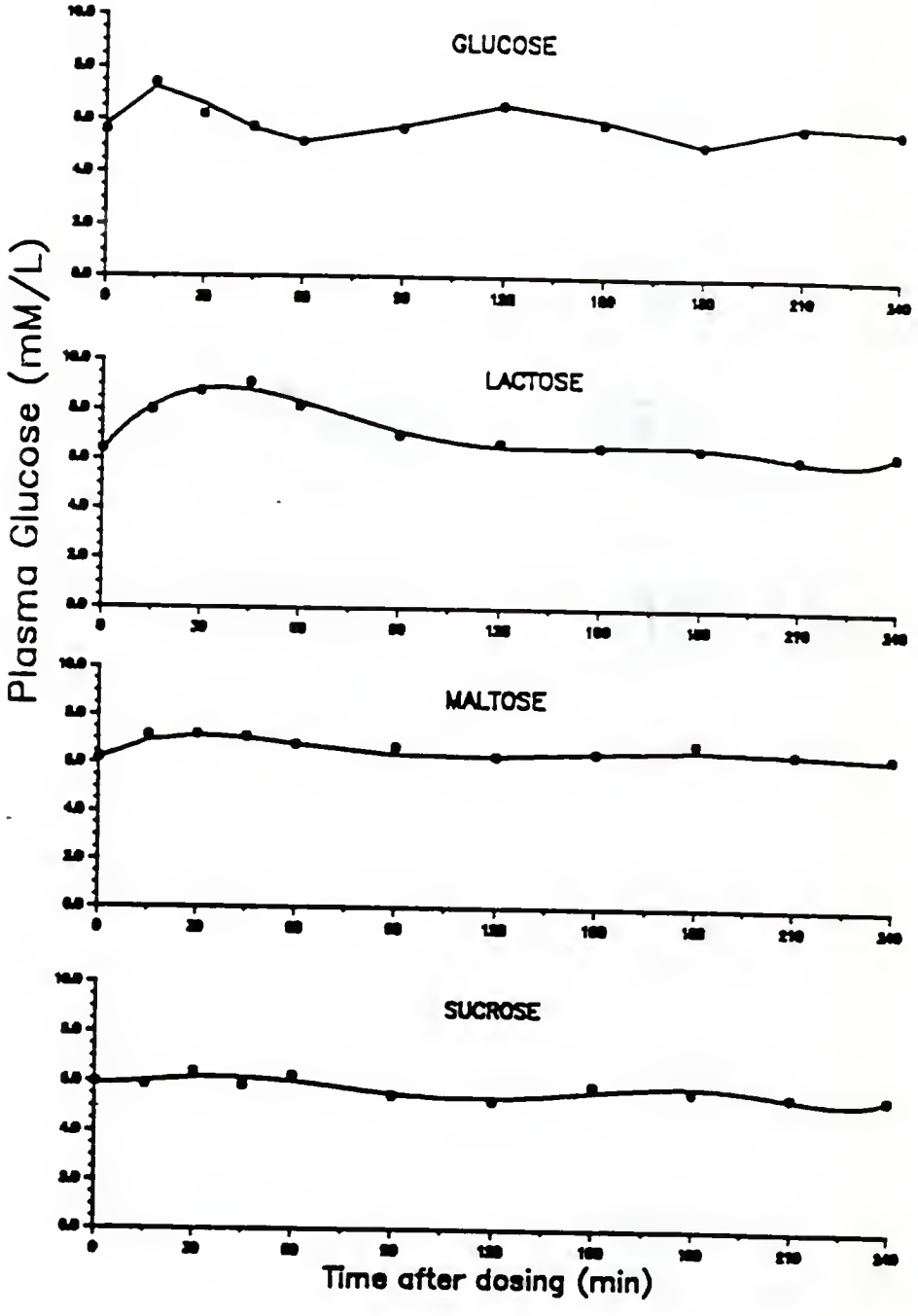

Figure 3.4. Changes in plasma glucose (least squares means) over time in 3-day old foals fed either glucose, maltose, lactose, or sucrose.

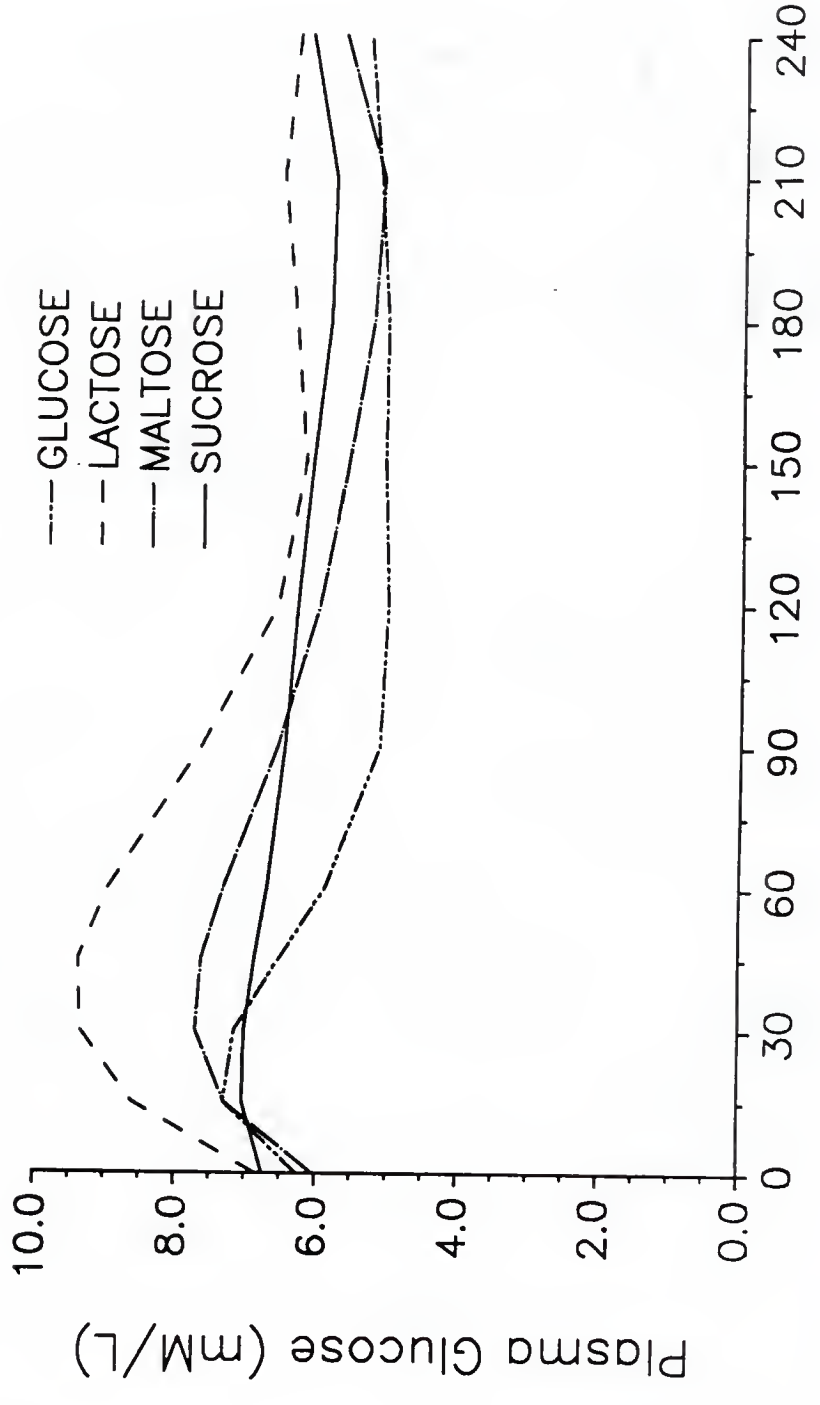

Figure 3.5. Changes in plasma glucose over time in 5-day old foals fed either glucose (SE mean = .21), lactose (SE mean = .13), maltose (SE mean = .13), or sucrose (SE mean = .11)

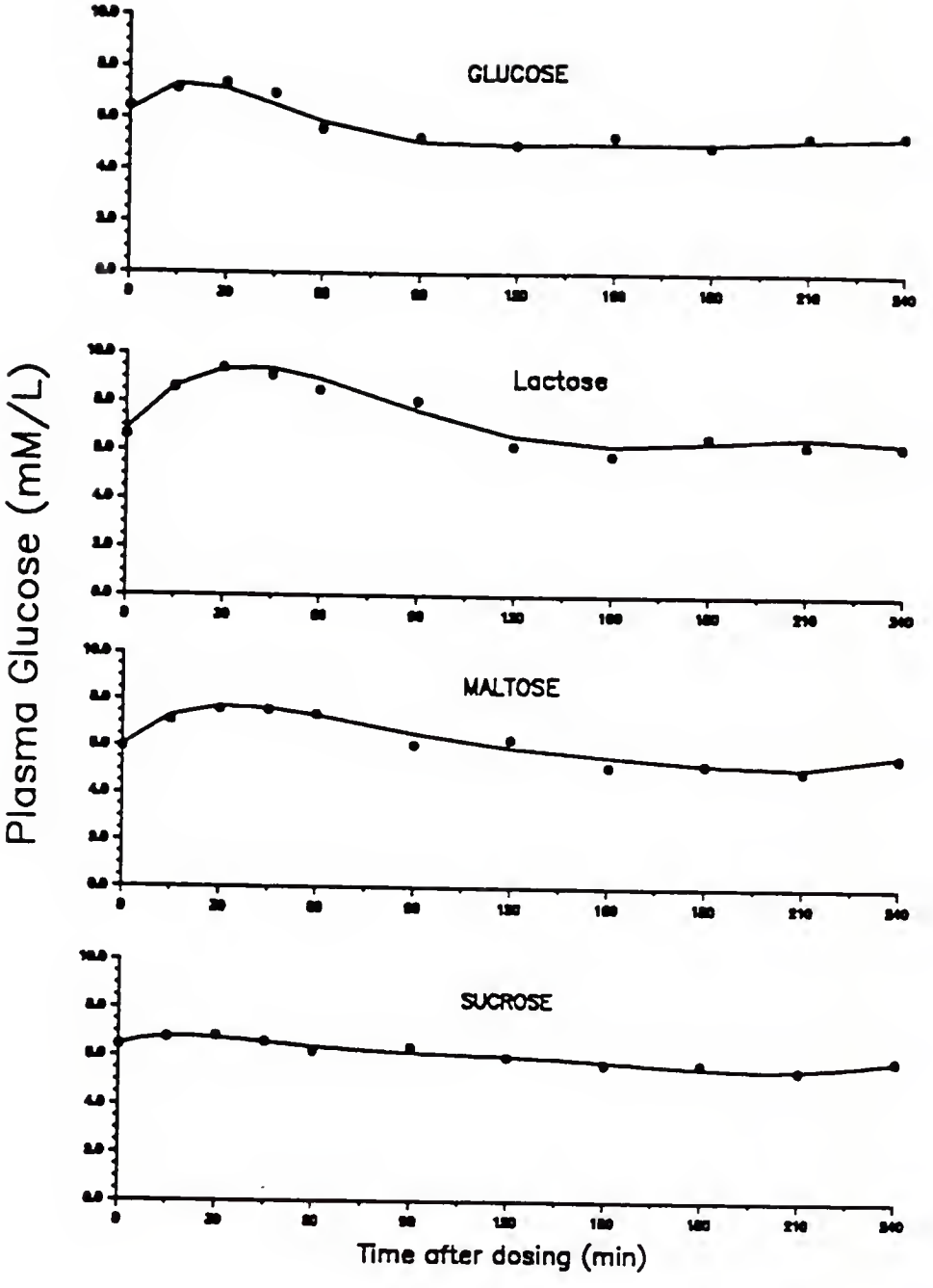

Figure 3.6. Changes in plasma glucose (least squares means) over time in 5-day old foals fed either glucose, maltose, lactose, or sucrose.

Discussion

Fasting Blood Glucose in Neonatal Foals

The glucose concentration in the blood of very young foals is higher than in the blood of older foals and adult horses (Becht and Semrad, 1985). The normal, resting blood glucose in the adult horse has been reported to be 4.77 ± .61 mM (Roberts, 1975). Kitchen and Rossdale (1975) reported glucose concentrations of 5.3 ± .97 mM within the first 5 min after birth and 4.6 ± .18 mM after 30 min in seven Thoroughbred foals. All of the foals had nursed by 1 to 2 h postpartum. At 14 h of age, these same foals had increased their blood glucose concentrations to 7.3 ± .69 mM. Glucose concentrations for foals from birth to 12 h old range from 6.4 ± .8 to 8.0 ± 1.6 mM (Rose et al., 1979; Bauer et al., 1984), respectively. Bauer et al. (1984) determined serum glucose in foals to be 9.2 ± 1.6 at 1 d of age, 9.3 ± 2.0 at 3 d and 9.0 ± 1.0 mM by 7 d. Rose et al. (1979) reported somewhat lower values. Foals sampled had concentrations of 8.0 ± .6 at 12 to 36 h and 8.4 ± .4 mM during wk 1 to 4. Many factors could be responsible for the difference values reported. One important factor is time after nursing. None of the workers noted the elapsed time from the last nursing episode to sample collection. Even though foals nurse frequently, this may affect reported concentrations. The foals used in this

experiment were fasted for 2 h starting at 6 h postpartum, before the treatment solution was administered (time = 0).

On d 1, the 2 h fast caused a considerable decrease in blood glucose. Even though the mean was 4.14 ± .27 mM, six of the 13 foals had values of 3 mM or below. By d 3, the foals were able to maintain higher fasting glucose concentrations. The foals may have become better at regulating glucose via insulin/glucagon interactions, more efficient at gluconeogenesis or better able to spare glycogen stores with increased utilization of lipid stores. Perhaps some or all of these systems are not fully functional at birth. All of the foals were born on or within a few days of the calculated foaling date, so prematurity could not be a factor when evaluating the effect of fasting.

The mean fasting blood glucose concentrations for d 3 (6.08 mM) and d 5 (6.31 mM) were higher than the reported resting concentrations for adult horses (4.77 ± .61 mM) by Roberts (1975). This is explained by the major energy sources utilized in each stage of a horse's life.

Lactose, absorbed as glucose and galactose from the small intestine, is the major energy source for the very young foal. As the foal matures, an anaerobic microbial population becomes established in the cecum and large intestine. As in the rumen, the fermentation process produces volatile fatty acids (VFA). These are absorbed and

are an important source of energy, along with glucose from carbohydrate digestion in the small intestine. Consequently, the adult horse has a higher resting blood glucose than ruminants which rely heavily on VFA for energy, and lower than nonruminants which rely mostly on small intestinal absorption of glucose. For example, normal resting blood glucose in cattle is approximately 2.78 mM (Bergman, 1970), while the value for adult swine is reported to be 5.56 mM (Tumbleson and Schmidt, 1986).

Treatment Curves Pooled Over All Days

The purpose of this trial was to determine the extent to which very young foals would be able to digest carbohydrates that may be included in neonatal enteral formulas. It was presumed that oral glucose would be absorbed easily and the peaks in blood glucose produced would provide a reference curve. It also was presumed that normal, healthy foals would be able to digest lactose. The resultant curves also would be used for reference. The data suggested that while oral administration of glucose and lactose result in increased blood glucose in the young foals, the pattern of absorption differed. The test for homogeneity of the two curves shows they are not parallel. Glucose treatment resulted in short, steep peaks occurring shortly after time = 0. In contrast, oral lactose resulted

in large peaks occurring later after administration and remaining high longer than the glucose treatment peaks.

In studies on adult horses, Roberts (1975) found high peaks in blood glucose associated with galactose administration. Galactose was only detected in the plasma with high oral doses. Roberts suggested that in the horse, galactose is rapidly converted to glucose in the liver, and then released into the circulation. Very young foals were not included in Roberts' study. However, data from the present trial would suggest this also may be the case in neonates. Future investigation of disaccharide digestion in the foal might compare the results of lactose, glucose, and galactose tolerance tests.

It was not known prior to the trial if neonatal foals had measurable sucrase or maltase activities. The activities of these enzymes in newborn intestinal mucosa varies with species. For example, human infants are capable of digesting both of these disaccharides at birth (Paige and Bayless, 1987), but calves and piglets are not (Code, 1968; Manners and Stevens, 1972; Ahrene et al., 1969).

The sucrose tolerance curves remained almost flat throughout the trial suggesting that sucrase activity was extremely low. The M curve produced from from data taken over all 3 d was not parallel to the pooled S curve. The differences in the treatment curves pooled over all days

suggests that there may have been some hydrolysis of maltose during the trial.

To further investigate the differences in absorption between the treatments, it is helpful to compare treatment-day curves.

Day 1 Treatment Curves

On the first day of extra-uterine life, oral glucose and lactose administration resulted in peaks in plasma glucose. However, the two curves were not parallel. The lag time before the peak in the L curves may be due to the time required for hydrolysis of the disaccharide, or the hepatic conversion of galactose to glucose, which requires a series of four reactions.

The S curve decreased slightly after time = 0, remaining almost flat during the trial period. The M curve, rising only slightly during the 4 h, was different from the S curve. It was also different from the glucose and lactose absorption patterns. The d 1 data suggest that oral glucose and lactose solutions are absorbed readily, but sucrose and maltose solutions are not.

Day 3 Treatment Curves

The L and G curves d 3 curves are similar to the d 1 curves, except that the peaks occur earlier in the post-administration period. Perhaps time and colostrum ingestion

have resulted in increased digestion and absorption efficiency. In piglets, puppies, and infants, colostrum intake has a profound effect on the digestive tract, resulting in increased length, weight, and absorptive surface area, as well as stimulating the development of enzymes (Goldstein, 1985; Widdowson, 1985; Widdowson, 1984).

Three-day old foals seem better able to withstand a short fast, with less decline in blood glucose, than 1-d old foals. As suggested earlier, perhaps the liver is now more efficiently using gluconeogenic pathways or mobilizing lipids to spare glycogen. Insulin and glucagon secretion may be more finely tuned to maintaining blood glucose concentrations than they were at birth. The older foals may have increased glycogen and fat reserves. Further investigation will be necessary to determine which mechanisms are involved.

On d 3, the M curve rose slightly during the first hour. The curve was not demonstrated to be statistically different from the G curve. This may be due to the fluctuations seen in the right hand portion of the G curve, although a characteristic glucose-treatment peak occurred during the first 30 min. The reason for occurrence of these fluctuations is not known. Glucose determinations were repeated on these samples to confirm the results.

On d 3, the M curve was not parallel to the L curve. The large peak produced from the lactose-treatment data does

not occur in the M curve. Therefore, maltase activity in the small intestine of these foals was extremely low.

Day 5 Treatment Curves

Although the plasma glucose curve after oral lactose administration contains a large peak during the first 90 min after treatment and the G curve shows a smaller peak of short duration, the curves were not statistically different ($P > .05$). The M curve, almost intermediate to the G and L curves especially during the first 90 min, could not be shown to be statistically different from either of the two curves. The S curve appears similar to the d 1 and d 3 curves. The sucrose tolerance curve, although almost flat, was not statistically different from any of the pooled G, L, or M curves ($P > .05$).

On d 5, the changes in plasma glucose after oral glucose and lactose administration are similar to those observed on d 1. It is not surprising that lactase is very active in the newborn and that the products of hydrolysis, glucose and galactose, are readily absorbed. It has been suggested that in the horse, the rate of galactose absorption may exceed that of glucose (Roberts, 1975). This trial was not designed to determine absorption kinetics. However, on all days the glucose-treatment peak appeared sooner after dosing than the peak in blood glucose following lactose treatment. It is not known wether this lag period

is due to increased absorption time or to hepatic conversion of galactose to glucose.

Sucrose does not appear to have been absorbed during this trial. This agrees with Roberts et al.(1974), who reported very low sucrase activity in the small intestine of newborn foals. The activity increased during the first year, reaching adult concentrations by 7 mo of age. In the present study, dosing with sucrose on d 1 and 3 did not result in an increase in sucrase activity on d 5. Dollar and Porter (1957) could not detect sucrase activity in neonatal calves, even with sucrose feeding. Sucrase activity does not appear in calves younger than 44 d old (Huber et al., 1961). Although piglets utilize glucose and lactose equally well at birth (Dollar et al., 1957; Ahrene et al., 1969), sucrase activity does not occur until after the first week (Huber et al., 1969).

On d 1, maltose administration resulted in a slight rise in plasma glucose concentration, but no peak was observed. On d 3 and 5, a slight peak was observed. It is possible that maltase becomes active near the end of the first week. Roberts and coworkers (1974) obtained equine intestinal tracts from slaughterhouses. They detected only low maltase activity in fetuses close to the end of gestation. The activity increased during the first month after birth. No data were given for the very early neonatal period.

Other species also have limited maltase activity shortly after birth. Piglets are not able to digest maltose until the end of the first week (Cunningham and Brisson, 1957). Maltase activity could not be stimulated in the intestine of calves during the first 44 d (Huber et al., 1961).

Because it was shown in calves (Dollar and Porter, 1957) and foals (Roberts, 1975) that the results of oral tolerance tests correlate well with tissue analysis for determining relative enzyme activities, some conclusions can be drawn concerning maltase, lactase, and sucrase activities in healthy foals. Lactose, the normal substrate presented to the intestinal mucosa in newborn foals, was well digested and absorbed. Sustained peaks in plasma glucose were produced from the data for each day, shortly after the treatment was administered. It is likely that other suitable substrates would have an absorption pattern similar to that of lactose or glucose.

Low maltose digestion and the lack of sucrose digestion, even on d 5 of the trial, was evidenced by the lack of substantial peaks in the plasma glucose curves. This suggests that maltase and sucrase activities are not developed sufficiently in foals of this age to support the use of these sugars in enteral formulas for neonatal foals less than 1 wk old. Because the inclusion of glucose or other monosaccharides increase the osmolarity of the diet,

they must be used with caution. The choice of a carbohydrate source is a difficult, but important decision. Therefore, to discover an ideal energy source for compromised neonates, other carbohydrates need to be investigated in future studies.

CHAPTER IV
CONCLUSIONS

The use of animal models for investigation of diseases in humans has been evaluated and debated extensively. Realistically, an alternative to performing invasive studies with humans, especially infants, is needed to provide basic information. While no model is perfect, it can be argued that swine are similar to their human counterparts in many respects. The colostrum-deprived neonatal piglet model provides many opportunities for basic research in nutrition. The model may also be used to substitute for foals for many of the same reasons. Piglets are small, tractable, and adaptable to laboratory conditions. As a result of this study, techniques imployed to perform surgery and care for the piglets were improved and can be used in future trials.

The low survival rate of the Group E piglets fed the elemental diet did not allow comparison of the two lipid sources. The piglets apparently lacked sufficient maltase activity to digest the carbohydrate source of the diet. Published data, as well as information obtained from this study, suggest that maltase activity is low compared with lactase, during the first week of life. Isoflurane

anesthetia greatly improved the post-surgerical recovery of the piglets in comparison with ketamine anesthesia, and is recommended for future studies.

Techniques for mixing formulas, performing surgery, and raising colostrum-deprived piglets were improved during the course of this study, and should benefit future trials.

Newborn, as well as 1 wk old sow-fed piglets, had numerically higher lactase activitiesin the small intestine compared with sucrase and maltase activities. Sow-fed and Group C piglets that did not ingest maltose had detectable maltase activity in the small intestine. Similarly, the Group E piglets that had not ingested lactose had detectable lactase activity on d 7.

The piglets kept on trial for 7 d had longer and proportionally heavier small intestines than did piglets killed at birth. This is probably due to the effects of age and feeding.

It was not clear from published reports if very young foals had adequate maltase activity. Because the development of disaccharidases before and after birth varies with species, it was not possible to predict the activities of these enzymes in foals during the first few days postpartum. Before engaging in a feeding trial using the Peptamen formula on foals, it was necessary to determine their ability to digest this maltose.

The results of the foal study suggested that foals 3 d old and older have an improved ability over newborn foals to maintain normal blood glucose concentrations after a short fast.

Foals as young as 1 d postpartum are able to absorb glucose and digest lactose. Maltose is digested only slightly by foals on d 3 and 5. The maltase enzyme becomes active too late in life for a maltose-containing formula to be fed shortly after birth. Sucrase was not digested to any appreciable degree even in 5-d old foals. On the basis of the oral tolerance tests given to foals up to 5 d old, maltose would not be a suitable carbohydrate source for inclusion in an enteral formula. Sucrose would not appear to be a suitable substitute. Glucose was readily absorbed, but large amounts of this monosaccharide in formulas greatly increases the osmolarity. This may not be tolerated well by compromised neonates. The results of this foal trial indicate that glucose from lactose administration was well absorbed. However, prematurity or damage to the intestines may result in reduced lactase activity in neonates. Therefore, alternative carbohydrate sources appropriate for compromised neonates need to be investigated in future trials.

APPENDIX A
PIGLET AND FOAL DATA

Sucrase activity in proximal, middle, and distal sections of the small intestine of piglets fed an elemental diet for 7 days

Piglet	Date of Birth	Sucrase Activity μMoles glucose produced/hour/gram wet tissue TI	MI	BI
P172	Mar 2	0	0	0
P173	Mar 2	0	0	0
P218	Sep 1	3.5	0	0
P222	Nov 30	2.2	.8	.4
P226	Nov 30	.6	2.4	1.3

Maltase activity in proximal, middle, and distal sections of the small intestine of piglets fed an elemental diet for 7 days

Maltase Activity
μMoles glucose produced/hour/gram wet tissue

Piglet	Date of Birth	TI	MI	BI
P172	Mar 2	43.0	53.4	156.9
P173	Mar 2	107.2	43.6	17.2
P218	Sep 1	25.7	59.6	31.4
P222	Nov 30	35.3	64.9	53.3
P226	Nov 30	21.0	35.2	23.4

Lactase activity in proximal, middle, and distal sections of the small intestine of piglets fed an elemental diet for 7 days

Lactase Activity
μMoles glucose produced/hour/gram wet tissue

Piglet	Date of Birth	TI	MI	BI
P172	Mar 2	104.0	189.2	555.7
P173	Mar 2	310.3	163.8	133.4
P218	Sep 1	90.7	238.9	90.2
P222	Nov 30	102.7	231.1	187.7
P226	Nov 30	47.5	47.4	37.8

DATA FROM PRELIMINARY FOAL TRIAL

DAY 1		DAY 5[a]	
TIME (mins)	PLASMA GLUCOSE (mM/L)	TIME (mins)	PLASMA GLUCOSE (mM/L)
0	7.18	0	8.71
15	8.36	50	8.37
30	4.29	65	7.91
45	3.30	80	7.37
60	6.62	95	7.08
90	2.16	125	6.18
120	4.49	155	6.97

[a]On day 5, problems with the catheter prevented the collection of blood samples at the prescribed time. Therefore, the times listed are not the same as day 1 collection times.

LEAST SQUARES MEANS OF PLASMA GLUCOSE OF FOALS RECEIVING ORAL GLUCOSE, LACTOSE, MALTOSE, OR SUCROSE ON DAY 1 POSTPARTUM

TIME	Lactose	Glucose	Maltose	Sucrose
(mins)		(mM/L)		
0	4.77	3.28	3.77	4.81
15	5.34	5.12	4.19	3.38
30	6.34	6.34	4.37	3.43
45	6.99	6.41	5.03	3.40
60	7.78	5.82	5.38	3.36
90	7.99	3.89	5.41	3.32
120	6.92	3.39	5.30	3.35
150	5.70	2.65	5.14	2.80
180	4.97	3.28	5.20	2.99
210	4.68	2.41	5.20	3.23
240	5.11	2.82	5.29	3.12

LEAST SQUARES MEANS OF PLASMA GLUCOSE OF FOALS RECEIVING ORAL GLUCOSE, LACTOSE, MALTOSE, OR SUCROSE ON DAY 3 POSTPARTUM

TIME	Lactose	Glucose	Maltose	Sucrose
(min)		(mM/L)		
0	6.40	5.65	6.24	5.97
15	8.01	7.39	7.16	5.91
30	8.76	6.21	7.20	6.40
45	9.11	5.71	7.11	5.88
60	8.18	5.16	6.79	6.24
90	7.02	5.72	6.71	5.49
120	6.70	6.58	6.34	5.28
150	6.56	5.92	6.49	5.89
180	6.49	5.12	6.88	5.65
210	6.11	5.78	6.50	5.50
240	6.35	5.65	6.42	5.47

LEAST SQUARES MEANS OF PLASMA GLUCOSE OF FOALS RECEIVING ORAL GLUCOSE, LACTOSE, MALTOSE, OR SUCROSE ON DAY 5 POSTPARTUM

TIME	Lactose	Glucose	Maltose	Sucrose
(min)		(mM/L)		
0	6.63	6.43	5.95	6.44
15	8.58	7.14	7.12	6.77
30	9.39	7.36	7.60	6.83
45	9.10	6.97	7.56	6.59
60	8.49	5.61	7.38	6.21
90	8.07	5.31	6.09	6.34
120	6.21	5.01	6.31	5.97
150	5.81	5.36	5.19	5.70
180	6.56	4.96	5.27	5.69
210	6.25	5.35	5.00	5.46
240	6.23	5.42	5.58	5.87

APPENDIX B
DISACCHARIDASE ASSAY PROCEDURE

PROCEDURE FOR DETERMINATION OF LACTASE, SUCRASE, AND MALTASE ACTVITY IN INTESTINAL TISSUE

This assay is designed to measure disaccharidase activity in intestinal tissue by measuring the amount of glucose produced when the tissue is incubated with different disaccharide substrates. Maltose, sucrose, and lactose are the substrates used in this trial. When one molecule of these sugars is cleaved by the appropriate enzyme, one molecule of glucose is liberated. The exception is maltose, which releases 2 glucose units. Therefore, the amount of glucose produced by the maltose incubation must be halved to calculate the true activity present. After incubation with the substrate for 30 minutes, the Trinder reagent is added and incubation continues for an addtional 30 minutes. The Trinder causes the glucose to be oxidized to gluconic acid and peroxide. The peroxide then reacts with 4-Aminoantipyrine and p-Hydroxybenzene sulfonate and the peroxidase enzyme to form a colored compound (Quinoneimine Dye). The color intensity, which is read at an O.D. of 505nm, is proportional to the amount of glucose produced. The amount of enzyme present intestinal tissue varies with species and age. Therefore, the amount of tissue homogenate to be assayed has to be determined for each type of tissue analysed. This is done by assaying the tissue using different concentrations of homogenate in the incubation tubes. The amount is varied until the amount of glucose

produced is within the values of the standard curve and in detectable amounts.

Preparatory work:

1. Allow spectophotometer lamp to warm up, if necessary.
2. Heat water bath to 37°C.
3. Place Trinder reagent in a beaker and dH_2O in a beaker and place in a covered bucket of ice next to the bath. This allows time for the Trinder undissolved solids to settle and for the water to become cold.

Equipment needed:

1. 12x75 mm tubes for incubations.
2. 13x100mm tubes for substrate and sodium phosphate buffer.
3. 9" Pasteur pipettes with rubber bulb for homoginization procedure.
4. Pipettes.

Preparation of Homogenates from piglet tissue:

Immediately after a piglet was euthanized, the intestine was removed and carefully dissected from the mesentary. The intestine was cut into 3 segments of equal length. The total weight of the empty intestine was obtained. The proximal, middle, and distal thirds were frozen and later assayed separately.

To reduce sampling errors, the segment of intestine to be assayed was cut into small pieces, from which random samples were taken. This was done while the tissue was partially thawed, but kept cold. A 1 gram sample produces enough homogenate to perform the disaccharidase, protein, and DNA assays. A 1:5 w/v homogenate is prepared by adding 5 ml of the homogenizing buffer to a 1 gram tissue sample. Tissues were homogenized thoroughly in a hand glass on glass homogenizer, keeping the tissues, buffers, and homogenizers cold at all times.

The sodium-phosphate buffer and substrates were pipetted into the test tubes prior to adding the homogenate. The tubes were incubated immediately. In this trial, duplicates of each substrate-tissue combination were assayed with 2 different amounts of homogenate.

Assay procedure:

Several preliminary assays were done to determine the relative amounts of each of the enzymes present, This was done to determine the amount of homogenate to be added to the incubation tubes that would result in detectable O.D.'s within the standard curve.

Each assay should include reagent blanks, containing only reagents and no homogenate, and tissue blanks, containing only buffer and homogenate, but no substrate.

1. Added to each tube:

*the appropriate amount of homogenate (according to the level of enzyme activity present) and .05M sodium phosphate buffer to total 375 μl.

*300 μl of a substrate (except for "tissue blanks"). Buffer is added to tissue blank tubes in place of substrate to determine the amount of glucose present in the sample.

2. Vortex tubes. Incubate tubes at 37^{o}C for 30 minutes.

3. Add 375 μl cold Trinder reagent. Incubate 30 minutes at 37^{o}C.

4. Add 750 μl of cold water to stop the reaction.

5. Read tubes at O.D. 505nm on spectrophotometer. Using a batch sampler facilitates reading tubes in a short period of time after the addition of the water. Color is stable for at least 20 minutes.

Calculations:

Standard Curve:

1. Calculate linear regression line for standard curve. $y = mx + b$ where y is the absorbance, m is the slope, x is the glucose concentration, and b is the y-intercept. Use the linear regression coefficients to calculate the amount of glucose produced in the sample tubes.

2. Average replicated sample O.D.'s. Subtract the reagent blank O.D. from these average O.D.'s. The glucose in each tissue is then calculated using the regression equation determined by the standard curve.

3. The substrates maltose, sucrose, and lactose may contain free glucose. The tissue samples will also have some free glucose. The amount of glucose from these sources must be calculated and subtracted from the amount of glucose produced by disaccharidase hydrolysis. The tissue blanks run with the sample (no substrate added) allow for the determination of glucose in the sample of intestine. Subtract the calculated glucose in the tissue blanks, the reagent blanks, and the standard curve blank from the calculated glucose in the sample. The result is the glucose produced by enzyme hydrolysis (corrected gluocse). 4. Divide the corrected glucose (μg) by the amount (μl) of homogenate used. If different concentrations of homogenate were used, average the values obtained from each set.
5. For maltase activity, divide the glucose concentration (μg/μl) by 2 for all tubes containing maltose. The units are μg glucose formed/hr/μl homogenate.
6. μg glucose/μl homogenate = mg glucose/ml homogenate

Y μM glucose/mg protein/hr =

X μg glucose/μl homogenate *

$$(1 \div .180)(1/\text{g tissue/g homog})\left(1/\frac{\text{mg protein}}{\text{g tissue}}\right)$$

(The formula weight of glucose is 180 g/mole.)
The results can be expressed as μM glucose produced/hr/gram tissue or on the basis of amount of protein or DNA in the tissue.

Prepartion of Buffers

0.5M stock solution of $NaH_2PO_4.H_2O$ (monobasic) FW=137.99

Dissolve 68.995g in deionized water (dH_2O). Bring volume to 1 liter.

0.5M stock solution of Na_2HPO_4 (dibasic) FW=141.96

Dissolve 70.983g in dH_2O. Bring volume to 1 liter.

Homogenization buffer (0.01M sodium phosphate buffer, pH 6.0, .002% Triton X-100)

0.01M dibasic solution

Mix 20 ml of the 0.5M dibasic stock solution with 980 ml dH_2O

0.01M monobasic solution

Mix 20 ml of the 0.5M monobasic stock solution with 980 ml dH_2O

Adjust the pH of the monobasic solution to pH 6.0 with the dibasic solution using a pH meter.

0.05M sodium phosphate buffer pH 6.0 (need a minimum of 400 ml to prepare disaccharide solutions)

0.05M dibasic solution

Mix 100 ml of the 0.5M dibasic stock solution with 900 ml dH_2O

0.05M monobasic solution

Mix 100 ml of the 0.5M monobasic stock solution with 900 ml dH_2O

Adjust the pH of the monobasic solution to pH 6.0 with the dibasic solution using a pH meter.

0.5M sodium phosphate buffer pH 7.0

Adjust the pH of 500 ml of the monobasic stock solution to pH 7.0 with the dibasic stock solution using a pH meter.

1.0M Trizma base stock solution

Dissolve 60.55g Trizma in dH_2O. Bring volume to 500 ml.

1.0M Trizma hydrochloride stock solution

Dissolve 78.8g Trizma HCl in dH_2O. Bring volume to 500 ml.

1.0M Tris buffer pH 7.0

Adjust the pH of 200 ml of the Trizma HCl stock solution to pH 7.0 with the stock Trizma base solution.

Preparation of Substrates

0.188M lactose in 0.05M sodium phosphate buffer pH 6.0, α-lactose monohydrate, crystalline FW=360.32

Dissolve 6.774g in 0.05M sodium phosphate buffer. Bring final volume to 100ml. Keep on ice or refrigerated. Do not use after 1 week.

0.0156M maltose in 0.05M sodium phosphate buffer pH 6.0, maltose, crystalline hydrate FW=360.32

Dissolve 0.562g in 0.5M sodium phosphate buffer. Bring final volume to 100 ml. Keep on ice or refrigerated.

Do not use after 1 week.

0.375M sucrose in 0.05M sodium phosphate buffer pH 6.0, sucrose, crystalline FW=342.30

Dissolve 1.284g in 0.05M sodium phosphate buffer. Bring final volume to 100 ml. Keep on ice or refrigerated. Do not use after 1 week.

Preparation of Trinder Reagent

Combine 50 ml of 1M Tris buffer pH 7.0 and 50 ml of 0.5M sodium phosphate buffer. Add to this the contents of 1 vial of Trinder reagent (Sigma). Stir for 30 minutes. Allow solution to settle. Use supernatant only. This reagent is light and temperature sensitive. Store in a dark container on ice or in cold room.

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BIOGRAPHICAL SKETCH

Lori Pamela Rice was born March 13, 1957, in Cleveland, Ohio. She lived and attended school in Lyndhurst, Ohio, graduating from Charles F. Brush High School in 1975. She then entered the zoology program at The Ohio State University, as a direct-admission honors student, receiving a B.S. in 1979 with a combined animal science-zoology major. During her undergraduate program, she worked at various veterinary clinics involving large and small animal practices and became a registered animal health technician. After graduation, she accepted a position as head laboratory technician with a large pediatric practice.

In 1981, after having taken several courses locally at Cleveland State University, she returned to The Ohio State University. She completed the requirements for an M.S. degree in animal nutrition in 1983. Her master's thesis involved culturing and identifying types of anaerobic bacteria from the cecum and colon of ponies fed different diets.

In 1984, she was accepted as a doctoral student in the University of Florida College of Agriculture. Her educational program included equine, ruminant, and human nutrition, reproductive and general physiology, and biochemistry. The research program emphasized neonatal

nutrition, equine nutrition, energy metabolism, and biochemistry.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Edgar A. Ott, Chairman
Professor of Animal Science

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David K. Beede
Associate Professor of Dairy Science

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Sandi Lieb
Associate Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Edward L. Johnson
Associate Professor of Animal Science

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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